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STUDIES ON SEX REVERSAL IN AMBLYSTOMA: XII. STERILITY AFTER REVERSAL OF OVARY TO TESTIS IN THE AXOLOTL

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A. INTRODUCTION

The development of functional testes in *Amblystoma tigrinum* following reversal of sex (female to male; Humphrey, '31) led to the hope that offspring might be secured from matings of such animals with normal females. *A. tigrinum*, however, proved to be poorly adapted to breeding experiments under laboratory conditions. The writer therefore undertook to repeat the experiment in sex reversal with the axolotl (*A. mexicanum*), since animals of this species can be bred under laboratory conditions without necessity for duplication of the conditions for hibernation essential with a terrestial species. Although it was found that reversal of ovary to testis could be accomplished without great difficulty in the axolotl, the resulting males, with few exceptions, were found incapable of reproduction. The developmental-anatomical basis for this condition constitutes the subject matter of this report.

B. MATERIALS AND METHODS

The axolotls used were from stock originally furnished by the Morris Biological Farm of the Wistar Institute.[1] Both the dark axolotl and the white or partial albino strain have been utilized.

Reversal of ovaries to testes was induced by subjecting them throughout development to the influence of a testis, the latter organ being introduced by orthotopic transplantation of a gonadal preprimordium[2] in embryonic stages (28 to 32 of Harrison's series; for technique consult Humphrey, '29). The sex of the grafted animals was determined by exploratory laparotomy at from four to nine

[1]The writer wishes to express his gratitude for the coöperation of Dr. Cranford Hutchinson of the Wistar Institute.

[2]I.e., the gonad developed from the graft replaces the right gonad of the host animal.

months after grafting. The incision for this was made on the left side to permit thorough inspection of the gonad of the host. If this proved to be an ovary of the inhibited or freemartin type, in which reversal (formation of testicular lobules or tubules) was not yet apparent, further laparotomies were performed at intervals of a few months until the occurrence of reversal had been demonstrated. When reversal was deemed sufficiently advanced, the graft testis which had induced it was usually removed. The animals were subsequently mated with normal axolotl females until their sterility appeared clearly established, after which they were autopsied and preserved for study.

Although the possibility of intermixture of donor and host tissues on the unoperated (left) side following orthotopic transplantation cannot be excluded, objection to this method of inducing sex reversal is weakened by the fact that the changes secured by its use can be duplicated by other procedures (parabiosis, ectopic grafting) not open to the same objection (Humphrey, '36, '38). Moreover, since the axolotl ovary in animals carrying testis implants is usually without demonstrable testicular structure for several months after grafting, it would seem probable, when testis lobules or tubules finally appear, that such gonads are not mosaics of donor and host tissues, but ovaries in which an actual reversal has been induced.

C. Results

1. *Frequency of Reversal in the Axolotl Ovary*

Because of the possibility of an incomplete removal of the primordial germ cells in preparing the site for orthotopic implantation, reversal of sex will be considered as having occurred only in those cases in which the ovary of a female host became transformed into a testis under the influence of a testis graft. This graft in the earlier experiments was homoplastic (i.e., from an axolotl donor), but when it was found that the axolotl testis inhibited ovarian development less effectively than that of *A. tigrinum,* heteroplastic transplantation was employed with embryos of the latter species as donors.

In Table 1 is presented the sex distribution in the seven groups of grafted axolotls in which sex reversal was secured. Although the excess in number of ♀♀[3] combinations may be in part the result of a selective mortality, there is histological evidence that in Group 2 at

[3]In the text and in Table 1 the first symbol of any pair indicates the sex type of the gonad derived from the graft, the second symbol the sex of the host.

TABLE 1

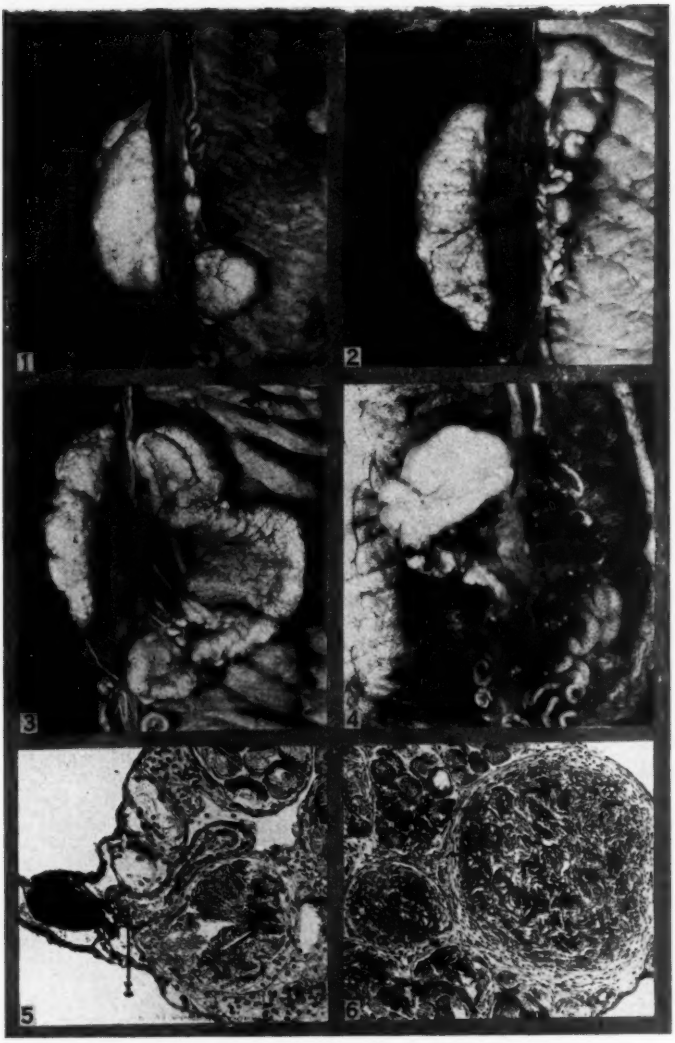
SEX DISTRIBUTION AND REVERSAL IN GRAFTED AXOLOTLS

The first symbol indicates the type of the gonad derived from the graft.

Group No.	Host	Donor	♂♂	♀♀	♀♂	♂♀	♂♀ Hosts with reversal
1	Dark Ax.	Dark Ax.	4	5	5	2	1
2	" "	" "	13	20	9	7	3
3	" "	A. tig.	3	12	3	6	2
4	" "	" "	9	18	3	11	3
5	White Ax.	" "	5	4	3	4	4
6	" "	" "	4	1	3	3	3
7	" "	" "	3	3	5	3	3
		Totals	41	63	31	36	19

least it is the result of reversal of testes to ovaries in ♂♀ or ♀♂ combinations, a reversal which tends to be relatively complete by the time the animals are old enough for exploratory laparotomy. A similar reversal in the testis of the axolotl host in ♀♂ combinations in Group 4 is also highly probable, and would explain the excess of ♀♀'s and the small number of ♀♂'s in this series. The occurrence of such a reversal, however, or the character of the sex ratio in unoperated animals, has little or no bearing upon the question of reversal of axolotl ovaries to testes in the ♂♀ combinations. In these the reversal is either so delayed in its occurrence, or is so incomplete in its earlier stages, that the original or genetic character of the ovary can be ascertained beyond doubt at the first laparotomy and its transformation to a testis readily followed by subsequent exploration.

In the 36 ♂♀ animals a partial or complete transformation of the ovary to a testis occurred in 19 cases. Of the remaining 17, two showed the ovary not reduced to the atrophied state which ordinarily precedes reversal, one had the ovary dominant and the testis in reversal, and 13 died or were otherwise disposed of before reaching the age of six months. In only one animal kept to an age of over a year did the ovary, if reduced to the atrophic state, fail to show testicular lobules. In the white axolotls of Groups 5, 6, and 7, the ovaries in all 10 ♂♀ animals underwent reversal, a 100 per cent record not obtained in any other experimental series or with any other species of Amblystoma. This result is probably in considerable part due to the effective inhibition of the ovarian cortex by the *A. tigrinum* testis grafts carried by these animals. With such inhibition of the ovary its reversal may be expected in practically all animals before they reach the age of 18 months, and frequently before the end of one year.


1
2
3
4
5
6

FIGURES 1-6

Figures 1 to 4 are photographs (x 1.75) of the gonads of axolotl hosts, in situ, exposed by removal of the gastro-intestinal tract and other viscera. The cephalic end is uppermost in each case, and the graft testis is at the reader's left. Graft testes, except that of Figure 4, are from *A. tigrinum* donors.

1. No. 1456, age 13 mo. The gonad of the host (at right) had undergone a relatively early reversal at its caudal end, the testis lobe there having been seen at exploration at 8.5 mo. The series of small lobes ahead of it are of recent development.

2. No. 311, age 28.5 mo. The gonad of the host (at right) is slender and irregular in its caudal part due to emptying of many of its lobules. The gonad was entirely ovarian on exploration at 5 mo. but showed small lobules present a year later.

3. No. 312, age 28 mo. The testis which has developed from the ovary of the host (at right) is of particularly large size in this animal. Reversal had probably begun some little time after the first exploration (at 5 mo.), since it was still incomplete one year later.

4. No. 191, age 22 mo. The graft testis in this animal (from an axolotl donor) had failed to suppress development of the ovary of the host, which shows numerous pigmented ova; a small testis lobe which developed in this ovary between the 10th and 15th months had subsequently degenerated. The graft has become an ovotestis; note scattered pigmented ova in its slender caudal portion.

Figures 5 and 6 are photomicrographs of transections from the testis of No. 156, age about 29 mo. This testis had arisen by reversal of the left ovary, after which the graft testis had been removed.

5. Hilar portion, showing in transection the longitudinal duct, which had been injected with India ink by way of the vas deferens. Note that the ink did not enter the intragonadal duct (I) adjacent to the hilar duct; this intragonadal duct contains many spermatozoa. x 80.

6. A large sac (at right) formed by distention of one of the lobules (tubules) of the testis with spermatozoa forced into it from the intragonadal duct system; at its left is a sac reduced to an almost solid state by destruction of the spermatozoa it once contained, and above and below this are normal lobules of mature spermatozoa. x 50.

2. *Pattern of Reversal*

While in some axolotl ovaries the development of lobules may occur more or less simultaneously throughout the organ, it would appear that in the majority they first develop in one or more limited regions or lobes, somewhat as in *A. tigrinum* (Humphrey, '31, '38). Eventually, however, lobules appear in other regions, often giving the gonad a distinctly segmented effect (Figure 1). These lobes or segments later fuse, but the testis thus arising continues to be of irregular form as lobules (tubules, ampoules) empty or degenerate in one region and develop in another. This irregularity in form is often in marked contrast with the more regular contours of the *A. tigrinum* graft (Figures 2 and 3).

The removal of the graft testis favors the continued growth of the reversed organ, which may sometimes become greatly convoluted in form due to its continued increase in length in comparison with the extent of the mesorchium attaching it. In one instance such a testis attained a length of 65 mm. in an animal of 29 months from which the graft had been removed about a year previously. Even in the presence of the graft testis however, the transformed gonad may attain a size fully as large or even larger than a normal axolotl testis, as illustrated in Figure 3. In general appearance, too, such testes may be indistinguishable from the normal.

3. *Permanency of Reversal*

The ovarian cortex in the axolotl is apparently more resistant to the inhibitory action of the testis than is that of the ovary in *A. tigrinum* or punctatum. Even in the presence of a tigrinum testis, small numbers of oöcytes may escape degeneration and grow to maturity, despite the fact that the ovary has at the same time become more or less completely transformed into a testis. The further differentiation of young oöcytes, however, is effectively checked as a rule either by the tigrinum graft or, after its removal, by the fully transformed ovary (now testis throughout), and the gonad thus eventually ceases to contain ova of any stage of development.

The inhibition of the ovary by an axolotl testis is often insufficient to prevent the development of considerable numbers of mature pigmented ova. In such cases the animal becomes an adult hermaphrodite; animals of this character have been treated more fully in another report (1941). In some instances in such hermaphrodites, oöcytes are permitted to develop even from the persistent male cortex of the

graft testis, converting it into an ovotestis (Figure 4). Before the persistent character of the axolotl ovarian cortex was fully appreciated, the graft testis was removed rather promptly from animals in which the ovary had begun to show development of testicular areas. In several of these axolotls the cortex promptly hypertrophied and eventually brought about the complete destruction of those testicular lobes which had developed, the gonad thus becoming a normal ovary of mature type. The discovery of this in one or two animals led to exploratory laparotomies in others before the degeneration of the testicular lobes had proceeded to completion. In these, extirpation of the ovarian part of the gonad was undertaken. This was apparently quite incomplete in one instance, in which the testicular lobe completely disappeared during the next four months. In another animal a remnant of the ovarian cortex gave rise to between 125 and 150 mature ova which finally degenerated, although delaying spermatogenesis in the large testis, in which no further development of oöcytes occurred. In two other animals extirpation of the ovarian part of the ovotestis was followed by marked hypertrophy of its testicular component, and no ovarian tissue was present at autopsy 7 and 18 months later.

In a few instances a small number of mature ova or pigmented corpora atretica have been found along the free margin of a gonad which otherwise had become entirely testicular in structure. The absence of younger oöcytes in such gonads may be taken as indication that they would have remained testes indefinitely.

In view of the facts presented above, it may be concluded that reversal of ovaries to testes in the axolotl is permanent if the persistent ovarian cortex is not permitted to hypertrophy due to a premature removal of the graft testis. It is believed that the tendency of the ovarian cortex to persist and hypertrophy is in no sense dependent upon the female soma of the host since it is noted in ovarian grafts in male hosts as well. While the use of an *A. tigrinum* testis to inhibit the ovary has proven more effective, permanent reversal through the use of axolotl grafts could doubtless have been obtained in a higher percentage of cases had their action been allowed to continue for a longer period of time.

4. *Sterility after Reversal*

Many animals in which the ovary had undergone reversal were tested as breeders, either before or after removal of the graft testis. This was done by placing the animal to be tested in an aquarium with

an adult female, all animals of the colony at other times being kept singly in small individual aquaria. The two were placed together in the evening and left together only until the following morning, at which time the aquarium was examined for spermatophores. These were carefully inspected to determine the presence or absence of spermatozoa. All matings in which sterile spermatophores were deposited are recorded in Table 2. The spermatophores in each instance

TABLE 2

DEPOSITION OF STERILE SPERMATOPHORES BY AXOLOTLS FOLLOWING REVERSAL OF HOST OVARY AND REMOVAL OF GRAFT TESTIS

Group No.	Animal No.	Date	Number of spermatophores
1	91	2-15-35	several
	"	3-11-35	2
	"	5-20-35	not recorded
5	154	1-28-39	2
	"	2-21-39	2
	"	3-13-39	several
	"	3-25-39	"
	"	5-17-39	7
	"	2-11-40	5
	"	4- 8-40	2
5	156	1-31-39	3
	"	3- 3-39	6
	"	3-31-39	6
	"	4-13-39	5
	"	5- 7-39	3
5	158	1-15-39	5
	"	3- 5-39	7
5	200	1-28-39	2
6	311	2- 9-40	6*
6	312	4-26-40	1*

*Spermatophores sterile, although the graft testis was present as well as the testis derived from the ovary of the host. Testes derived from *A. tigrinum* grafts in axolotl hosts commonly show a disturbance in spermatogenesis such that few or no spermatozoa are discharged into the vas deferens after the animal's first spermatogenetic cycle.

consisted only of the transparent gelatinous base, the opaque white, apical mass of spermatozoa of the normal spermatophore being absent.

Until 1940 no records were made of those tests which did not result in deposit of spermatophores. It is probable, however, that such animals as No. 154 and No. 156 gave as high a percentage of mating responses in 1939 as did normal males in the breeding colony. In 1940, No. 154 deposited spermatophores twice in 22 trials, in favorable comparison with a semicastrated male of the same age (three

years) which deposited fertile spermatophores twice in 19 trials. In the same period Nos. 311 and 312 gave but one positive response each in 7 and 19 trials respectively, and the third reversed male of Series 6 gave no response in 11 trials. These three animals compare very unfavorably with a semicastrated male of the same series and age (two years) which deposited fertile spermatophores in 7 out of 12 trials made over the same period. It is uncertain, however, whether occasional control males might not have given equally few positive responses, since the number of such males in the colony was too small to establish the range of variation with regard to mating reactions.

In the normal white axolotl male, spermatozoa may be found in the ductus deferens through several months of the year. Fertile spermatophores have been deposited from early January until late June by males in the writer's colony, and it is probable that spermatozoa may be found in the ducts in some individuals for a considerable period before and after these dates. In at least six of the sex-reversed animals autopsied during spring months the size of the testis and the condition of the cloaca would have led one to expect to find spermatozoa in the vas deferens. In these and all other animals in which the testis had arisen by reversal, however, no spermatozoa were ever found in the vas at either exploration or autopsy at any time of the year. This is in harmony with the fact that only sterile spermatophores were ever deposited by any of these animals after removal of the graft testis.

5. *The Testis and Its Duct System*

In gross appearance the testes as a rule presented no striking abnormalities. Their lobules were never dilated into large fluid-filled cysts as is frequently the case in testes derived from heteroplastic grafts (*A. tigrinum* or punctatum) in axolotl hosts. In some instances, however, there were found protruding from the surface small opaque sacs which were found on puncture to be solidly packed with masses of spermatozoa. Some of these sacs bulged only slightly above the surface and were scarcely larger than normal lobules, but a few were pedunculated and from 3 to 5 mm. in diameter. Similar structures were never seen in normal or semicastrated males.

Since it was quite evident that spermatogenesis had proceeded to completion following sex reversal, the failure of the spermatozoa to reach the vas deferens raised the question whether, due to the late development of the testis from an ovary there had not been some fail-

ure of development of its efferent duct system. At autopsy, however, especially after fixation, it was evident that the testis possessed efferent ductules, three or more in number, and that longitudinal anastomeses between these had usually formed a Bidder's canal close to the genital portion of the mesonephros, in the fashion pictured by Rodgers and Risley ('38) for normal Amblystoma males. Connections of this Bidder's duct or canal to the mesonephros, and numerous connections of the latter to the wolffian duct or vas deferens were also distinguishable. No part of this duct system between testis and vas deferens was ever found distended with spermatozoa.

The small size of some of the passages as they appeared under low magnification at autopsy suggested they might not be patent. To test this point India ink was injected into the caudal end of the vas deferens in one specimen (No. 156) before fixation, and was observed to flow through the mesonephros and enter the testis by way of at least six efferent ductules. In serial sections of the testis, however, it was found that the ink which appeared to have entered that organ was really confined to a longitudinal duct lying along its attached edge or hilus (Figure 5) either in the mesorchium or at most only a short distance within the testis itself. The intragonadal duct system did not communicate with this hilar longitudinal duct, which on the other hand, was continuous with the several efferent ductules.

The intragonadal ducts in this animal, which was killed in late June, contained numerous spermatozoa, which had apparently entered them in the normal fashion from emptying lobules, many of which were in evidence. From these ducts the spermatozoa had apparently been forced into occasional lobules which had undergone distention to form the sac-like structures already mentioned. Some of these were doubtless of recent origin, since their appearance, aside from their size and the crowded arrangement of the spermatozoa, resembled that of the normal lobule. In many, however, the sac had acquired a greatly thickened wall (Figure 6) as a result of the proliferation both of the sustentacular cells of the lobule and of the surrounding stromal elements. While the latter gave rise to an external covering of connective tissue, the former proliferated as irregular anastomosing columns or cords of epithelial cells which penetrated into the periphery of the mass of spermatozoa. The latter being gradually destroyed by phagocytosis, the greatly thickened walls of the sac finally remained as an almost solid mass reminiscent of the corpus luteum of a small mammal. Various stages in the regression of these epithelioid bodies were ob-

served, including small acidophilic masses comparable to a small corpus albicans of the ovary.

In addition to the testis above described (that of No. 156), several others were serially sectioned, either completely or in part. In these it was found that when few or no lobules had emptied, and the intratesticular ducts were not distended with spermatozoa, no sperm-filled sacs were to be found. The occurrence of these sacs in four different animals, however, indicates that their formation is a common mode of adjustment to the abnormal pressure set up by lobules emptying into a closed duct system. It permits the emptying, regression, and regeneration of numerous lobules, and so doubtless plays an important part in maintenance of the spermatogenetic and endocrine functions of the organ.

Two of the reversed gonads examined in complete serial sections (those of Nos. 311 and 312; Figures 2 and 3) were found in agreement with No. 156 in lacking connections of the intragonadal ducts to the efferent ductules or the longitudinal hilar duct with which these are continuous. In No. 311 only three efferent ductules were present and a longitudinal duct was found joining two of these; the entire cephalic half of the testis was without hilar or efferent ductules, except for occasional short segments of such ducts completely isolated in the mesorchium or at the hilus. In No. 312, eight efferent ductules were identified, with a hilar duct present in a large extent of the testis. In both these animals some of the intragonadal ducts contained spermatozoa; in both, these ducts were easily traced and their lack of continuity with the hilar duct or efferent ductules readily demonstrated. It may be concluded therefore, that the sterility which commonly follows reversal of ovary to testis in the axolotl has an anatomical rather than a physiological basis, and that this basis is a lack of continuity between the efferent ductules and the intratesticular portion of the duct system with which the lobules or tubules directly communicate (tubuli recti and rete testis, essentially).

In two instances axolotl testes which had arisen by reversal from ovaries were found on sectioning to have connections between the intragonadal ducts and the efferent ductules, although these connections were few in number. In neither animal, however, had such a stage of development been reached that lobules had emptied and spermatozoa reached the vas deferens.

One of these potentially fertile animals is No. 1456, which died at approximately 13 months of age. The cephalic two-thirds of the left

gonad, which had been of ovarian structure on exploration at 8½ months, at death consisted of a series of three small testicular lobes (Figure 1), whose lobules were still in early stages of development. Throughout this region, connections of the intragonadal ducts to the efferent ductules or hilar longitudinal duct were entirely lacking. In contrast with this, the large caudal lobe of this gonad (in which lobules had developed before the laparatomy at 8½ months), had a number of intragonadal ducts opening to the longitudinal duct which lay at its hilar border; this latter duct, in turn, was connected to the mesonephros by four efferent ductules. The second potentially fertile animal, No. 1430, belonging to the same series (Group 7; see Table 1), was killed at 14 months. In this animal, as in 1456, a large testicular lobe had developed at the caudal end of the ovary, whose cephalic end was still partly of ovarian structure. A smaller testis lobe of later origin joined the cephalic end of the large one. In the latter the longitudinal duct, which was continuous with two efferent ductules, took a course deep to the hilus and made connections with a small number of intragonadal ducts, although other such ducts often ended blindly in its vicinity.[4]

6. *Ovary Before or During Reversal*

In some axolotl ovaries, within a few months after grafting, the cortex was much inhibited, only a few enlarged oöcytes remaining; and ovarial sacs were lacking, although the epithelium of the sac could be recognized as groups or strands of cells in the medulla. Sometimes a longitudinal duct was present at the hilus with varying numbers of germ cells associated with it. Such modified ovaries are little different from those of *A. tigrinum* in which an early reversal (at two to five months) is of frequent occurrence in animals of the Chicago strain. To determine the condition of the duct system after early reversal in the latter species, the region of the ovary showing lobules was examined in serial sections in 10 animals selected at random from the many cases available. In every instance, one or more efferent ductules could be found to connect to the intratesticular ducts (rete) either directly or by way of a longitudinal hilar duct. Presumably, in an axolotl ovary undergoing a similar early reversal, a like condition in the duct system would obtain; this would account for the two potentially fertile animals already described.

[4] A third animal of this group has been successfully mated; the character of its offspring has been reported elsewhere (*Anat. Rec.*, **82**, *Suppl.*, p. 77; 1942) This animal is still living.

Many axolotl ovaries under testicular dominance, however, were less completely inhibited. Ovarial sacs sometimes developed and numerous oöcytes enlarged. A longitudinal hilar duct was often absent, or if present, had no germ cells associated with it. Specimens of such ovaries obtained by biopsy or at autopsy from animals of eight months or more have shown numerous large oöcytes and traces of ovarial sacs. By this time, however, the sac remnants were usually not found near the hilus, but more distally, associated with the persisting large oöcytes, and were without connection to a hilar duct if the latter were also present. In some parts of such ovaries, as the large oöcytes degenerated, duct-like structures appeared, sometimes seen developing as buds from the epithelium of the ovarial sac reflected over a large oöcyte. These ducts became associated with gonia, to be seen at times in cords, crowded in from the cortex. In this way the primordia of testicular lobules became established. Branching of the ducts which first differentiated served to establish other lobule primordia. Indifferent gonia remained in the germinal epithelium long after all large oöcytes had disappeared, and continued to be crowded into the underlying stroma to furnish the germ cells for additional lobules.

D. Discussion

To the writer's knowledge, the only instance of experimental sex reversal (ovary to testis) in the axolotl previously recorded is that reported by Witschi ('37) for a female joined in parabiosis to an hypophysectomized *A. tigrinum* male. In this axolotl the ovaries at one year were of the atrophic or freemartin type but on exploration and biopsy at 2 years 7 months they were found to be largely composed of testis tubules (lobules), with only a very few ova remaining. On autopsy at nearly four years of age the only remnants of the ovarian cortex were a few pigmented corpora atretica, the gonads, though very small and showing marked regressive changes, being otherwise of testicular structure. Witschi makes no reference to spermatozoa in the vas deferens, and suggests that the degenerative changes in the testes (formerly ovaries) may possibly be due "to the incompleteness of the efferent tubular system." Aside from these degenerative changes and the small size of the testes which developed (possibly due to an inhibition by the testes of the co-twin), the picture of sex reversal presented by this axolotl is in essential agreement with that found in many animals of the writer's series. The exact nature of the "incompleteness" in the duct system, however, was not stated.

A satisfactory explanation of the defective duct system found after sex reversal in the axolotl requires a brief account of the normal differentiation of the duct anlagen in the two sexes.[5]

In the early development of the gonad the rudiments of the future duct system of the testis appear as a number of cords or chains of cells (rete cords) extending from the mesonephric blastema into the gonad anlage. As the indifferent gonad undergoes modification in the male direction, that portion of the cord of cells within it proliferates to form the short tubuli recti to which the seminiferous lobules or tubules open, and the branching intratesticular ducts or rete tubules, running to the hilus. By longitudinal anastomoses the rete derived from one cord may unite with that originating from the others. Those portions of the original cords or duct rudiments lying outside the gonad become the efferent ductules which extend through the mesorchium from the testis to glomerular capsules of the genital portion of the mesonephros. Tubuli recti, rete, and vasa efferentia thus are in continuity throughout their development.

In the normal differentiation of the ovary from the indifferent gonad the rete cords do not proliferate to form a branching duct system as in the male. Instead, each cord hollows out to form a cavity, which by expansion transforms the cord into an extensive epithelial sac. By union of these sacs the ovary acquires a continuous cavity. The portions of the cords within the mesovarium are said to form functionless vasa efferentia in some urodeles but since the writer has been unable to identify such structures in axolotl females, it is assumed that they ordinarily degenerate in this species.

In the ovary of *A. tigrinum* which is brought under the dominance of a testis before the beginning of sex differentiation, either by parabiosis or by grafting of gonad preprimordia, the development of the rete cord into an ovarial sac as a rule, is completely suppressed. In regions of such ovaries which undergo an early reversal to testis, as frequently occurs in females of the Chicago strain, the cord instead develops into the branching duct system characteristic of the normal male, and retains its primary connection with the glomerular capsule. In regions in which few or no germ cells have become associated with the cords and no early development of testis lobules has occurred, the cords may be represented chiefly by a longitudinal duct formed by their anastomosis at the hilus of the gonad, with occasional duct rudi-

[5]A more detailed account, based on the work of various investigators, is given by Willier ('39).

ments extending farther into the medulla of the atrophic ovary. If scattered germ cells associated with these duct rudiments eventually increase and form testicular lobules, these lobules would drain either directly or by way of the longitudinal duct to the extra-gonadal portion of the cord which has persisted as an efferent ductule. Thus with an early and profound suppression of ovarian development, the rete cords are so modified as to provide a continuous duct system in regions in which a reversal of the ovary later occurs.

In the axolotl, as compared with *A. tigrinum,* the lack of continuity necessary for a functional duct system appears to result from a less effective suppression of ovarian development with a consequent delay in reversal. In such an ovary the rete cords may give rise to ovarial sacs, although these may not develop to the normal size. These sacs, however, or their epithelium if no lumen is formed, tend to lose their connections to the portions of the cords lying in the mesovarium, even though the latter, influenced by the graft testis, have been stimulated to persist as vasa efferentia. The epithelium lining the ovarial sac is of course homologous with that lining the rete and tubuli recti of the male. Eventually, remnants of this epithelium form duct rudiments which "attract" germ cells in gonial stages from the persisting cortex. Though the multiplication of these spermatogonia now produces testis lobules of normal structure, the intratesticular ducts (rete) to which they empty never regain the connections to the efferent ductules lost by the ovarial sac epithelium from which they were derived.

The explanation offered above is in no sense inconsistent with the fact that in two instances a continuity of the duct system following reversal has been demonstrated. In each instance this continuity was limited to a single region in which reversal had occurred relatively early, since in one animal lobules were seen on laparotomy at 8½ months, and in the other the region contained maturing spermatozoa at 14 months. The remainder of the gonad, in which reversal had evidently begun much later, in each case showed no connections between efferent ductules and intratesticular ducts. A similar delayed reversal appears to have occurred in a number of those animals which proved to be completely and permanently sterile.

Lack of continuity in the duct system not only precludes securing offspring of the reversed animals by natural matings, but prevents use of the "dry" insemination technique successfully employed by

Lipsett[6] and other investigators, since this requires seminal fluid obtained from the vas deferens. The insemination of unfertilized eggs of Amblystoma with a sperm suspension prepared by mincing the testis has proven unsuccessful, despite careful attention to the chemical composition and pH of the medium (Lipsett). It would appear, therefore, that hope of obtaining offspring from reversed axolotls lies chiefly in securing an early reversal such as had occurred in the two potentially fertile animals so far encountered, and in the one animal from which offspring have been secured (see footnote, p. 196).

The percentage of axolotl ovaries which may be caused to undergo reversal through the action of testis grafts compares favorably with that in *A. tigrinum* in which reversal of ovaries has been induced by parabiosis ('36) as well as by orthotopic and ectopic grafting ('31, '38). In *A. tigrinum* a considerable number of ovaries fail to undergo reversal even though kept under the influence of a testis well beyond their first year. In the white axolotls of Groups 5, 6, and 7 (see Table 1), in contrast, all ovaries became transformed to testes.

It is probable that the longer persistence of the ovarian cortex in the axolotl ovary under testicular dominance is an important factor in the high percentage of reversal secured and the rather complete transformation of the ovary to testis throughout its entire length. In ovaries the cortex of which quickly becomes reduced to a state of more or less complete sterility, any subsequent formation of testicular lobules must depend upon the presence in the medullary or hilar region of the gonad of germ cells which had become associated with rete elements there during earlier development. In the absence of such gonia, the cortex having become sterile, the ovary would remain permanently a thin flattened, sterile fold ("freemartin" gonad). If, on the other hand, germ cells of gonial stages remained in the cortex of the inhibited ovary, and were finally "attracted" into association with duct rudiments arising from the epithelium of the ovarial sacs, a reversal of the ovary might occur throughout a large part of its extent, even though in the testis thus formed the duct system might be defective. It is of course evident that long persistence of the cortex necessitates delay in removal of the graft testis even after reversal has begun, in order to preclude a possible cortical regeneration and the formation of an ovotestis.

[6]Lipsett, J. C., Department of Anatomy, School of Medicine, University of Maryland; personal communication, February, 1939.

E. Summary and Conclusions

1. Reversal of ovary to testis in the Mexican axolotl was readily induced by orthotopic implantation of gonadal preprimordia in embryos. Reversal, as a rule, occurred later than in *A. tigrinum* similarly grafted, but the entire gonad rather than a limited part usually became transformed to testis.

2. After complete reversal and the extirpation of the graft testis which induced it, the animals continued to exhibit the secondary sexual characters and mating instincts of the male, but except for those of one animal, all spermatophores deposited were sterile. At exploration or autopsy during the mating season the vas deferens was never found to contain spermatozoa, although these were present in great abundance in the testis.

3. The patency of the passages between the vas deferens and the testis was demonstrated in one animal by injection. In this and other animals, however, the efferent ductules were found to lack connections to those intragonadal ducts which correspond in position to the mammalian rete testis.

4. The lack of continuity in the duct system commonly found after reversal results from interruption of the primary continuity between the efferent ductules and the ovarial sac epithelium from which the intratesticular ducts are later derived. Since this interruption is probably due to the somewhat delayed reversal characteristic of the axolotl, an earlier reversal such as occurred in a few instances appears to offer the only hope of obtaining animals capable of reproduction.

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STUDIES ON THE EFFECTS OF X-RAYS ON REGENERATION IN THE FRAGMENTING OLIGOCHAETE, *NAIS PARAGUAYENSIS*

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A. INTRODUCTION

Within recent years numerous problems of development have been advantageously attacked, and our knowledge of them considerably extended, by the use of x-rays and radium. The application of this experimental technique has been particularly effective in the study of the cellular activities attending regeneration. The papers of Curtis ('36) and Butler ('34) may be referred to in this connection.

The present observations on *Nais paraguayensis* were made in conjunction with a study of the fundamental problem of the internal cellular manifestations of normal and x-rayed specimens, but since they concern chiefly externally observable phenomena they conveniently form the subject of a separate paper; the complementary histological analysis will be presented in a later publication.[1] Only such reference will be made here to this latter phase of the problem as is calculated to promote a clearer understanding of the purpose of the study.

As early as 1904, Bardeen and Baetjer found that regeneration in Planaria can be prevented by exposure to x-rays. In the same year, Schaper ('04) reported a similar inhibition of regeneration of the limb of Triton by exposure to radium emanations. Subsequently, a variety of animals, principally invertebrates, were investigated in reference to this phenomenon: Palmetohydra, Zawarzin ('29), Strelin ('29); Tubularia, Congdon ('12), Curtis and Ritter ('27); Planaria, Weigand ('30); Tubifex, Stone ('32); Lumbriculus, Zhinkin ('32), Turner ('34); Rynchelmis, Zhinkin ('34); Clavelina, Weigand ('30);

[1]This paper constitutes publication, in part, of a dissertation submitted to the Board of University Studies of The Johns Hopkins University in conformity with the requirements for the Degree of Doctor of Philosophy. The author wishes here to express his appreciation of the kindly and helpful criticisms of Prof. S. O. Mast, Dr. Gardiner Lynn, and Dr. Charles Brambel, during the course of the work.

Triton, Litschko ('30), Burnet and Scheremetjewa ('33); Amblystoma, Butler ('31, '33). In each, a similar inhibitive effect of radiation on regeneration was found to obtain. Complete references to these works may be found in the review of Curtis ('36).

In all species studied it seems clear that the clue to the inhibition lies in a selective injurious effect of the radiation upon those cells which normally are active in the regenerative process. And it appears that this effect is most likely to be interpreted in terms of the repeatedly demonstrated "Law" of Bergonié and Thribondeau ('06), according to which the sensitivity of cells to radiation varies directly with their reproductive capacity and inversely with their degree of differentiation.

Because of their remarkable regenerative capacities, together with their relatively simple metamerism, the oligochaetes have long been favored material in the analyses of restorative phenomena. The application of the radiation technique to this group has, however, been limited. Zhinkin ('32, '33), and Stone ('32, '34) first applied radiation to the study of oligochaete regeneration; they worked with Lumbriculus variegatus and Tubifex tubifex respectively. Turner ('34, '35) and Zhinkin ('34) similarly studied Lumbriculus inconstans and Rynchelmis limosella. In all, the internal changes accompanying the inhibition of regeneration were found to lie principally in the activities of the mesodermal "neoblasts" (or their parent tissues) and the ectodermal and endodermal epithelia.

Certain marked differences in the history of these regenerative materials (particularly the neoblasts, which have received most attention), the details of which need not be presented here, suggested the desirability of extending such an investigation to the family Naididae, the members of which are especially remarkable for their regenerative ability. Moreover, according to a brief statement of Randolph ('92), who originally described "neoblasts," in Lumbriculus, these particular cells are especially abundant in naids. *Nais paraguayensis* specifically recommended itself to the study since, according to Hyman ('38), the species constitutes the "one well established case among the oligochaetes of fragmentation as a regular method of asexual reproduction." And, although no detailed study had been made of its regenerative capacity, it is obviously great; growth is accomplished with extreme rapidity, and the species is readily cultivated.

Butler ('31, '33) was the first to employ localized irradiation in the study of regenerative phenomena; by this means he ('35) demon-

strated that the effect of x-rays in preventing limb regeneration in Amblystoma larvae is due to local action of the radiation on cells in the limb, and is not the result of general action of the radiation on the body of the animal as a whole. Further, his results were of significance in reference to the problem of the source of the cells upon which the regeneration of the limb depends, showing clearly that these cells must arise in loco, and are not brought to the site of regeneration from other parts of the body, as suggested by Hellmich ('31). Heretofore, so far as the writer is aware, all experiments on the effects of x-rays (or radium) on regeneration in oligochaetes have involved the exposure of the entire organism. And such appears to have been the case with all invertebrates studied excepting the single investigation of Puckett ('36) on Pennaria; he found, by means of regional irradiation, that x-rayed portions of colonies of this hydroid failed to regenerate, while protected portions regenerated new hydranths at normal rate, thus showing that the effects of the radiation are local so far as regeneration is concerned.

The threefold purpose of this paper is to present and discuss: first, observations on external features of fragmentation and regeneration; secondly, developmental manifestations of x-rayed material; and, thirdly, evidences regarding the modus operandi of the x-rays, and the local nature of the regenerative process.

B. Materials and Methods

Nais paraguayensis is not native to this country. It was obtained through the courtesy of Dr. L. H. Hyman of the American Museum of Natural History, New York City; she discovered it in aquaria there about seven years ago, and judges it was probably introduced therein with some South American fish or plants. The species was originally described by Michaelsen ('05). It reproduces asexually by fragmentation; this process consists, essentially, in the spontaneous separation of the animal into several more or less equal parts, without the formation of fission planes generally characteristic of oligochaetes. Aiyer ('24) first established the fact of its reproduction by this atypical method. The naid attains a length of 30-40 mm., and measures 0.3-0.4 mm. in width. The body is lightly flesh-colored and quite translucent. There is no information extant in reference to a sexual phase in its life history. It is easily cultivated on softened lettuce leaves suspended in shallow dishes of water (see Hyman '38).

The worms, generally anesthetized in 0.06 per cent chloretone,

were transected with no difficulty by rolling the blade of a small scalpel over them, against a glass surface. Although the anesthetic has no apparent effect upon subsequent regeneration, cut pieces were always thoroughly washed before being set aside for observation. Operations were made either with or without the aid of a microscope as the case required; anterior and posterior extremities are distinguishable from each other without the aid of a microscope.

The x-radiation experiments were begun at the Marine Biological Laboratory, Woods Hole, Mass., and continued in the Tumor Clinic of the U. S. Marine Hospital, Baltimore, Md.[2] At Woods Hole two Coolidge tubes were operated simultaneously, the material being "cross-fired" by two x-ray beams; the machine in Baltimore was a single tube arrangement of the usual therapy type. The radiation conditions were as follows:

Woods Hole		Baltimore	
KV	192	KV	250
ma	25	ma	30 cm.
dist.	33.2 cm.	dist.	21 cm.
rate	564 *r*/m	rate	467 *r*/m

For simple exposures the worms were placed in the basal portions of waxed paper cups or of small plastic drinking cups (cut to convenient size), containing 4-5 mm. of water or chloretone solution. Regional exposures were made after introducing the naids into fine glass capillaries, and protecting the desired portions with lead plate. Further details of this technique will be described and illustrated subsequently.

C. Observations

1. *Fragmentation*

The scope of this work does not include an analysis of the fragmentation process as such, but only in so far as morphological changes may be correlated therewith. While several of the present observations on this phenomenon constitute repetition of the work of Hyman ('38)[3] they were not undertaken for the express purpose of verifying

[2]The author wishes here to acknowledge his indebtedness to Dr. J. E. Wirth, *Director*, and Mr. J. E. Rose, *Physicist*, of the Tumor Clinic of the U. S. Marine Hospital in Baltimore, for the many kindnesses and facilities so graciously extended him.

[3]Her report, the first experimental work on *Nais paraguayensis*, was offered as a contribution to Child's theory of dominance and subordination and physiological isolation in relation to sexual reproduction, and was particularly concerned with factors controlling the time of fragmentation and the experimental alteration of the number of fragments into which the animal separates.

her findings; rather, it was felt that the difference in point of view justified their being repeated.

Upon attaining a length of 30-40 mm. *Nais paraguayensis* spontaneously separates (fragments) into several pieces, generally 5-8, without the previous formation of fission planes characteristic of oligochaetes; that is to say, regeneration ensues only after the separation of the several fragments. Regularly one to several constrictions of the body-wall are detectable along the length of the animal; these loci may persist for at least two hours. That these constrictions are the loci of ultimate separation was ascertained by mapping them prior to separation, and later finding them to correspond exactly to the points of separation. To all appearances the post-constriction stages of the process are chiefly physical and may be likened to the simple separation of a piece of wire following repeated bending at a particular point.

Of the several fragments produced, the head fragment is generally longest and composed of the largest number of completely formed segments; the remaining trunk and tail pieces are nearly equal in length, although, by reason of the decreasing size of the segments posteriorly, the tail fragments contain many more segments. All fragments quickly regenerate and grow to adult size, the head piece regenerating a tail, the tail piece a head, and the trunk fragments both a head and tail.

Fragmentation of adult worms can be induced within a day by isolating them in fresh water without food at 24°-27° C. The addition of a strand of surgical gauze, or some like material, about which the animal may twine is advisable. The readiness with which fragmentation occurs varies directly with the size of the specimen; this is shown in Table 1. Lower temperatures (circ. 15° C.) delay the process. Decapitation markedly accelerates the fragmentation of adult worms; this is shown in Table 2.

TABLE 1

EFFECT OF SIZE ON FRAGMENTATION-TIME OF ISOLATED WORMS

Temp. in degrees C.	Specimens length in mm.	Isolated number	Number of worms Fragmented on successive days after isolation 1d	2d	3d	4d	5d	6d	7d	Unfragmented on 7th day
24-27	10-15	32	0	1	1	5	4	4	1	16
24-27	15-20	38	1	5	14	10	2	4	1	1
24-27	20-25	26	6	13	3	3	0	0	0	1
24-27	30-40	36	31	5	–	–	–	–	–	0

TABLE 2

EFFECT OF DECAPITATION ON THE FRAGMENTATION-TIME OF 30-40 MM. WORMS

Number of worms	Number of fragmented specimens at times indicated, subsequent to isolation			
	7 hr.	12 hr.	24 hr.	48 hr.
Decapitated 38	10	38	—	—
Not decapitated 20	0	0	13	19

These observations on the whole confirm the findings of Hyman ('38), except as regards the loci of separation. From her statement (p. 127) that "the places at which fragmentation is to occur are not detectable by microscopic examination of the worms" it is not altogether clear that she had noted these loci. Their occurrence was of particular interest for the investigation at hand, since there was some reason to suspect that microscopic examination might reveal a condensation of regenerative cells, specifically neoblasts, at these points, such as is known to occur in the region of the fission planes of other naids. This matter will be dealt with in connection with the histology of regeneration.

2. *Regeneration of Cut Fragments*

Since no previous study had been made of regeneration as such in *Nais paraguayensis*, the extant information being limited to a few incidental observations (Hyman '38), the following study was made to ascertain the relative importance of several factors in the process, a knowledge of these factors being necessary in order that such manifestations may not be mistakenly attributed to experimental agents, which in reality are but the expression of limiting factors within the normal animal itself.

If cutting is accomplished neatly and quickly, post-operative mortality is negligible; the body-wall contracts immediately, drawing the cut edge together and forming a neatly rounded stump. Regardless of their size, cut fragments retain their original antero-posterior orientation in crawling or swimming. Unless otherwise noted, all work was carried out at average room temperatures.

In pieces approximating the size of natural fragments the first indication of head formation is apparent 8-12 hours after cutting; it appears as a small unpigmented mass of tissue (blastema), the extent of which is easily ascertained in contrast to the pigmented stump. The pigmentation of the stump is due chiefly to the brownish chlorogogue cells surrounding the gut, and it is convenient to measure new growth

from the cut edge of this chlorogogue layer, making allowance for the fact that it is slightly retracted from the wound surface. Within about 24 hours the postomium,[4] oral segment (Seg. I), and stomodeum are well formed anteriorly. At about 36-40 hours the first and second ventral setal bundles (on Segments II and III) and the cerebral ganglia are clearly visible. In 48-50 hours the complete head of five segments, with its entire complement of four ventral setal bundles (on Segments II, III, IV, and V) is regrown. At this stage, in addition to the setae, the principal organs to be noted are the prostomium, cerebral ganglia, muscular pharynx and a complex of cephalic blood vessels (Figure 3). At this time the head measures about 0.40 mm. in length, and though integrally formed it has not yet reached its definitive size; the head of a mature worm attains 0.63 mm. in length.

The early head and tail growths are easily distinguishable (Figure 10). Unlike the posterior growth, anterior regeneration involves the replacement of five and only five segments; these are termed the "cephalized" segments. The general term "cephalization," in reference to oligochaetes, indicates the specialization of a number of anterior segments. The term is, however, used in a special sense in reference to the Naididae; here, while the ventral setal bundles begin on Segment II (generally), the dorsal bundles begin only on Segment VI (occasionally more anteriorly). The first five segments, therefore, differ from the rest in having no dorsal setae; other lesser differences often obtain. Thus the head is a well-defined morphological unit.

The initial tail growth is apparent only slightly later than the anterior growth. While proceeding somewhat more slowly at first, it does not cease after the formation of a definite number of segments, but continues "indefinitely" until adult size is attained. In its earliest stage, like the head, it appears as a slight unpigmented growth. The anal segment is formed first, and this bears an easily distinguishable proctodeal invagination at about 15 to 20 hours. It is from immediately anterior to the anal segment that growth in length proceeds. The difference in pigmentation of the regenerate is equally as marked here as it is anteriorly (Figure 10), and remains apparent in some specimens long after the length of the regenerate has exceeded that of the original piece (Figure 9). Several dorsal and ventral setal bundles of the tail regenerate are apparent in the second day of growth, though they are not yet fully differentiated. The above fea-

[4]The prostomium of oligochaetes is not to be considered a segment; it corresponds neither in origin nor constitution with the body segments (Stephenson, '30).

tures of head and tail regeneration in transected pieces are identical with those occurring after natural fragmentation.

a. Regeneration along the antero-posterior axis. The question considered here is whether, in pieces approximating natural fragment size, regenerative capacity varies at different levels of the body, the capacity to be judged in terms of rate of growth. Adult worms (30-40 mm.) from the same culture were cut into approximately equal eighths, pieces from similar regions were kept together in stender dishes, at constant temperature, and the lengths of the anterior and posterior regenerates measured at definite intervals. Table 3 represents a typical experiment, and shows that in such pieces there is little difference in the rate of anterior and posterior regeneration at different levels; it further shows that head and tail replacement proceed independently of each other, the respective growths being practically the same in pieces where head and tail were regenerating simultaneously as in pieces where only a head or only a tail was growing (Pieces I and VIII). This does not mean to say that there is absolutely no gradient of regenerative capacity; the growth is so rapid that for a detailed study of the problem, the rate of growth would have to be considerably decreased by manipulation of the temperature, and the pieces cut smaller.

b. Regeneration from anterior and posterior extremities. The question considered here is that of the minimum head and tail piece length sufficient for regeneration. As Hyman ('38) reported, a head piece consisting of only the five cephalized segments will not regenerate a tail; at least three trunk segments must be left attached to the head in order that posterior regeneration might occur. Anterior extremities incapable of posterior regeneration will, however, regenerate anteriorly, and will also live in a tailless condition for some weeks. The regeneration of tail pieces of less than 13 segments was never observed. Some headless tail pieces of seven to nine segments remained alive for as long as a week; they become bloated and are definitely less viable than anterior extremities similarly incapable of regeneration. Heads are readily regenerated from newly regenerated tails provided, of course, the piece is sufficiently long.

c. Regeneration in relation to the size of cut fragments. The remarkable regenerative capacity of *Nais paraguayensis* is further revealed by the fact that pieces consisting of merely two segments will regenerate both anteriorly and posteriorly. Pieces of three segments regenerate thus quite consistently, but in two-segment pieces it more

TABLE 3

RATE OF REGENERATION (AVERAGE LENGTHS EXPRESSED IN MILLIMETERS) IN RELATION TO POSITION OF PIECES ALONG ANTERO-POSTERIOR AXIS (TEMP. CIRC. 24° C.)

Time interval after cutting in hours	Number of regenerating pieces measured	Designation of successive approximately equal eighths of adult worms													
		I	II		III		IV		V		VI		VII		VIII
		Post.	Ant.	Post.	Ant.	Post.	Ant.	Post.	Ant.	Post.	Ant.	Post.	Ant.	Post.	Ant.
26	30	0.14	0.18	0.13	0.18	0.19	0.22	0.14	0.21	0.18	0.25	0.21	0.25	0.19	0.24
72	30	0.81	0.48	0.83	0.42	0.75	0.44	0.76	0.43	0.90	0.46	0.85	0.44	0.89	0.43

often happens that head regeneration is incomplete (hypomeric) and tail regeneration fails completely. Only hypomeric heads and never posterior regenerates were found to develop in one-segment pieces. These and certain other data indicate that the anterior regenerate exerts an inhibiting influence on tail regeneration; it will be recalled that anterior regeneration proceeds slightly in advance of posterior growth.

Apart from the regenerative capacities of such very small pieces, size is a factor in the readiness with which regeneration takes place. This is clearly shown in Table 4 which represents a typical experiment

TABLE 4

THE GENERAL EFFECT OF THE SIZE OF CUT FRAGMENTS ON THE RATE OF REGENERATION IN *Nais paraguayensis* (TEMP. CIRC. 24° C.)

Time after cutting in hours	Number of of pieces of each size-group measured	Average length in millimeters of regenerated tissue.					
		3-5 seg. pieces		10-15 seg. pieces		20-32 seg. pieces	
		Ant.	Post.	Ant.	Post.	Ant.	Post.
50 hrs.	25	0.22	0.17	0.31	0.29	0.42	0.40

in this regard; the pieces were taken indiscriminately from all levels of the trunk, anterior and posterior extremities being discarded. Clearly, larger fragments are more favorable to the process. It will be clear that further differences in regenerative rate might be detected in pieces of different size from particular levels along the axis. Anterior and posterior regeneration occur repeatedly if newly regenerated portions are ablated from an original (15-20 segment) piece, after about two days of growth; this repeated growth can occur at least six times at either extremity, and probably more often, but, as might well be expected, the rate of growth decreases with successive ablations.

d. Regulatory phenomena. The term "regulation" in the sense in which it is to be understood here signifies an "adjustment" or modulation by which tissues undergo change in order to restore and maintain typical form. Two types of such regulation were observed in *Nais paraguayensis*, one ordinary, the other uncommon.

The ordinary type is as follows: In the adult animal the muscular pharynx is followed by an oesophagus; this is distinguishable from the more posterior gut region by being somewhat more narrow and exhibiting (most often) definite undulations in its restricted course of some six or seven segments. If an animal is severed, for example, through the midgut, the head regenerates but an oesophagus is lacking;

by way of adjusting this situation, the tissue of the midgut immediately posterior to the head becomes modified to form an oesophagus. This regulation of the gut tissue is discernible within a few days after complete restoration of the head. Thus is restored the post-cephalic specialization, typical of the organism, to meet the conditions of the new head. Such regulation does not occur in the absence of the head, as, for example, when the new anterior growth is repeatedly ablated. This type of regulation has been described in a number of oligochaetes, e.g., Stylaria (Harper, '04), Dero (Hyman, '16). On the basis of previous descriptions of other species, additional modification of this post cephalic region, especially as regards the circulatory system, most probably occur in *Nais paraguayensis*.

The second and uncommon type of regulatory phenomenon in *Nais paraguayensis* was observed in only two specimens. These two specimens were fragments resulting from the cutting of adult worms, one a trunk piece of eight segments and the other a tail piece of 20 distinguishable segments. In each the regenerated head grew at almost a 90° angle to the long axis of the body. The specimens thus restored were very active, but their locomotion was greatly impeded by this adverse position of the head. However, within about a week these heads had assumed their normal position in relation to the axis of the body, and the worms continued normally in their development. Harper ('04) reports a similar situation in Stylaria following upon oblique cutting of the body; attempts to reproduce this abnormality in *Nais paraguayensis* by cutting pieces obliquely were to no avail. These instances illustrate an interesting type of regulation, which, however, seems of less importance for developmental studies than the ordinary type previously described.

As previously indicated these observations on the general morphology of regeneration were undertaken as necessary and preliminary steps to the radiobiological and histological phases of the work; their detailed analysis belongs properly to a physiological study of the restorative process. It will be in point to mention here and now that the histological study has revealed that regeneration in *Nais paraguayensis* is accomplished principally through the activities of certain ectodermal, mesodermal and endodermal elements, much after the same general manner as described for other oligochaetes (see Stolte, '36); specifically the situation in this species is very similar to that described by Stone ('32, '33) for Tubifex tubifex, although, to mention one im-

portant difference here, in *Nais paraguayensis* the neoblasts are present as a persistent cell stock.

3. *Effects of Total Irradiation*

In view of the fact that x-rays inhibit regeneration in a variety of forms, it was reasonable to expect a similar inhibition in *Nais paraguayensis*. It was necessary, however, to ascertain the minimum total radiation necessary to effect this, thereby to eliminate as far as possible any other effects of the rays as would likely be accentuated by an amount in excess of the required minimum, or by a too rapid application of the same. While the experiments of Stone ('32, '33), Turner ('34), and Zhinkin ('34) offered some clue in this regard, it was found, in the beginning experiments, that under the conditions of the double x-ray tube at Woods Hole considerably smaller doses were sufficient for comparable inhibitions in the regeneration of *Nais paraguayensis*.

In several exploratory experiments, cut fragments of medium size, and whole worms, were exposed to various doses of radiation at different rates; some lots were anesthetized and others not. The exposures were as follows:

(*a*) 3,150 *r*/min. for 1, 2, 3, 4 mins. (3,150 *r* to 12,600 *r*)
(*b*) 2,500 *r*/min. for 0.25, 0.5, 0.75, 1.0, 1.25, 1.75, 2.0 mins. (625 *r* to 5,000 *r*)
(*c*) 564 *r*/min. for 2.5, 3, 4, 5, 6, 7, 8, 9, 10 mins. (1,410 *r* to 5,640 *r*)

The x-radiation inhibits regeneration, and the inhibition is roughly proportional to the total-*r* applied. With the higher rates of 3150*r*/min. and 2500*r*/min., the total-*r* necessary to effect a given degree of inhibition was somewhat less than was required with the lower rate of 564*r*/min. But by reason of a necrotic condition which these rates apparently emphasized, appearing externally as a destruction of the pigmented chlorogogue of the gut, their use seemed inadvisable. The rate of 564*r*/min. did not produce this necrosis so readily, if applied just long enough to preclude regeneration; some slight evidences of it appeared only a day or longer after exposure. This abnormality appeared also after a time in one isolated experiment in which a rate of only 150*r*/min. was used, in the application of 3000 *r*. With the selected rate of 564*r*/min. a six-minute exposure, 3384 *r*, completely inhibited posterior regeneration in practically 100 per cent of the specimens, and an eight-minute exposure, 4512 *r*, effected similar inhibition anteriorly. However, under the radiation conditions obtaining in Baltimore, where the rate was 467*r*/min., doses of 7000 *r* and 9000 *r* were required for similar posterior and anterior inhibition respectively.

These latter amounts correspond with the doses applied by Stone ('32, '33) and Turner ('34) in Tubifex and Lumbriculus. The difference in the two radiation conditions does not seem such as would in itself account for this considerable difference in the total-*r* required in each case; several possible explanations suggest themselves, but, pending further inquiry, the matter must rest for the time unexplained. These exploratory experiments indicate the importance of considering the rate at which x-rays are applied in such work; they suggest also that a quantitative radiobiological study could be pursued to advantage with this material.

It is of interest that a greater percentage of inhibition was obtained, both as regards its extent and the number of individuals affected, when the material was anesthetized than when it was not. Now while the anesthetic quiets the specimens, allowing, theoretically, a better distribution of the rays, the amount of absorption by such slight material is likely so small that this added susceptibility of the anesthetized worms would seem to be due almost entirely to the chloretone solution. Similar effects of other chemicals on the sensitivity of biological material to x-rays are known.

a. Anterior and posterior inhibition. Generally, adult worms were first cut into fragments and these subsequently exposed to the amount of radiation necessary to prevent regeneration; often, however, the adult specimens were first irradiated whole and then cut into pieces, any time from an hour to 36 hours later. No significant difference in the extent of inhibition was observed with these two procedures. To all appearances the process of healing in normal and x-rayed pieces is identical. As previously noted, the degree of inhibition is roughly proportional to the amount of radiation applied. Hypomeric heads, formed after smaller doses, generally bear a stomodaeal invagination. Complete anterior inhibition involves merely the formation of a terminal knob (Figures 7 and 8); these knobs generally persist for as long as the animal lives, but frequently become definitely less conspicuous; they often exhibit slight indications of a stomodaeum. With considerably more radiation, 12,000 *r* to 15,000 *r*, even the formation of these knobs is prevented. The characteristic post-cephalic regulation of the gut tissue, to form an oesophagus, does not take place when the formation of a head has been completely or almost completely inhibited. But of particular importance is the fact that inhibition of head regeneration requires a significantly higher dose of radiation than does posterior inhibition; the respective doses have already been indi-

cated. A one-minute exposure, at 2500*r*/min., was found particularly effective in producing specimens with definitely inhibited tails but with complete heads, although there is often considerable individual variation in response. The significance of this differential will be discussed in connection with the histological analysis, but it may be mentioned here that considered in the light of the generalization of Bergonié and Thribondeau ('06), referred to earlier, it suggests that anterior and posterior regeneration are accomplished by cellular elements exhibiting respectively different degrees of differentiation, head growth being effected, wholly or in part, by more highly differentiated cells than those responsible for tail regeneration.

Posteriorly there is the formation of similar "terminal knobs" (Figures 7 and 8); this term is taken from Stone ('32) who describes similar formations in x-rayed Tubifex. Definite indications of an anal opening are often found in these terminal structures; as with the cephalic knobs, their formation is prevented by excessively high doses of radiation. These anterior and posterior knobs have not the clear unpigmented aspect of newly regenerated tissue, and there are histological reasons for not considering their formation as true regeneration.

Some specimens with completely inhibited heads and tails will live for at least eight weeks, and with proper care they would probably live longer. Irradiated whole specimens do not recover their regenerative capacity, as revealed by their inability to regenerate when cut one to eight weeks after exposure; the same is true of irradiated fragments. X-rayed Tubifex lived for 147 days after exposure, after which time they were still incapable of regeneration (Stone, '32). The chlorogogue becomes increasingly necrotic with time, and the worms are reduced in girth; apparently starvation is the principal cause of death.

b. Effects of x-rays on fragmentation. The experiments undertaken in this connection were intended to answer only one question, namely, whether the destruction of the power to regenerate would preclude fragmentation. Accordingly, 48 adult (30-40 mm.) specimens were isolated in six groups of eight; each group received a different dose of radiation, as follows (rate 2500 *r*/min.): 2500 *r*, 5000 *r*, 7500 *r*, 10,000 *r*, 12,500 *r*, and 15,000 *r*. All worms were then isolated in the manner already described (temp. 22-24° C.). Within a day, at least two specimens of each original group of eight had fragmented into 4-6 pieces; at about two and one-half days all 48 worms had fragmented, each group averaging slightly more than six fragments per worm.

Thus for the present purpose the important fact established by this experiment is that the complete inability to regenerate does not prevent the animal's fragmenting.

4. *Regional Irradiation of Whole Worms*

Throughout this and other studies on the effects of x-rays on regeneration in oligochaetes, the question has continually arisen as to the manner in which the radiant energy exerts its inhibitory effect on the regenerative cells. The specific question in point has been well stated by Curtis ('36) who, writing of the effects of x-rays on oligochaete regeneration, observes: "Since it is a fair hypothesis that the chemicophysical changes at the cut surface may produce changes in the blood or coelomic fluid that might affect the reserve cells, one may question whether the effect of the radiation is upon a humoral activation of this sort or directly upon the reserve cells." Information in this regard is calculated to be of value not only in the further application of the radiation technique but also in various more properly physiological problems concerning the effects of x-rays on living systems.

Anesthetized adult worms were introduced into thin glass capillaries, of such internal diameter as would just conveniently accommodate them; they generally remain in a fixed position. At times, for purposes of orientation, it was convenient to stain specimens in a dilute solution of neutral red; the numerous coelomic fluid cells have a particular affinity for this dye, and the treatment does not interfere appreciably with regeneration. The worm-laden capillaries were then laid in grooves cut in a leucite plate (Figure 1), anterior-posterior orientation being noted; the plate, accommodating 11 capillaries, was cross-marked so that any movement of the worms during the experiment could subsequently be noted. Known portions of the worms were protected by slipping close-fitting lead plate over the capillaries. The capillaries and lead plates having been adjusted, the whole was transferred to the floor of an open-sided box which in turn was attached to the x-ray tube, in place of the therapy cone. The center and peripheral limit of the radiation field were indicated on the celluloid floor of the box, to secure conformity of exposure; by looking up through the floor of this arrangement, the original and post-irradiation positions of the specimens were checked. Subsequent to exposure the worms were returned to fresh water, and cut according to the plan indicated in Figure 2; controls consisted of worms similarly prepared

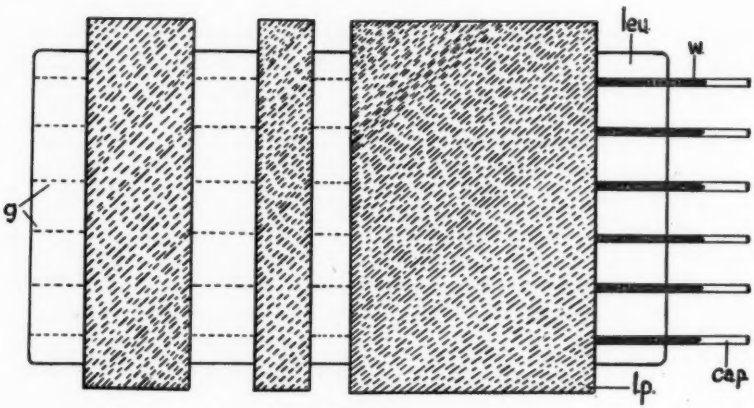


FIGURE 1

APPARATUS FOR REGIONAL IRRADIATION

The anesthetized worms (*w*) are introduced into fine glass capillaries (*cap.*); these are then laid in grooves (*g*) cut in a leucite plate (*leu.*), and the desired portions of the worms shielded with lead plate (*l.p.*). By various arrangements with lead plates of different widths, only three of which are illustrated here, the several types of exposures are made. The size of the specimens and capillaries are somewhat exaggerated here for the sake of clarity.

in capillaries, some of which were entirely protected from and others entirely exposed to the radiation. Although some preliminary experiments of this type were performed at Woods Hole, these were later repeated in Baltimore, and the present account deals with work done in the latter place; doses of 3000 *r*, 4000 *r*, 5000 *r*, 7000 *r*, 9000 *r*, and 10,000 *r* were applied.

Figure 2 illustrates schematically the several types of exposures, and Table 5 summarizes the results obtained, following the cutting of the worms in these respective regions. The demarcation between exposed and protected portions is not so precise as the diagrams portray, i.e., the edge of the lead plate does not mark exactly the limits of effective radiation, by reason of scattering of the rays. Further, the specimens are somewhat more contracted within the capillaries than when extended freely, just prior to cutting. With due attention to all factors, however, reasonably accurate distances were calculated. Under these conditions of glass encasement there was some, although not an unexpected measure of individual variation in response to a given dose of radiation. The results show that the irradiation of the several regions of varying extent and relationship, from one-sixth to five-sixths

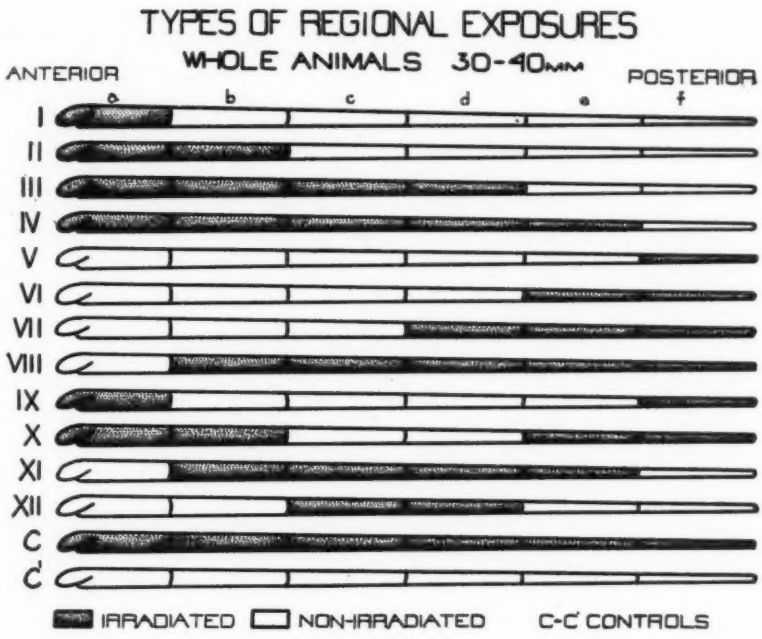


FIGURE 2

TYPES OF REGIONAL EXPOSURES OF WHOLE ANIMALS

See explanation in text, and summary of results in Table 5.

of entire specimens, inhibits regeneration in the exposed portions, but does not affect the restorative capacity of the protected portions of the same worms. These results lead to the conclusion that the effect of the rays is a direct, local one.

If the inhibiting effect of the radiation were borne to the regenerative cells indirectly as, for example, through some chemico-physical change effected in the blood or coelomic fluid, or through some hormonal disturbance effected in some other organ (e.g., nerve cord) and carried by the blood or coelomic fluid, and the circulation of these elements were continuous throughout the animal, it would be reasonable to expect that this inhibitory influence would be transmitted to regions of the worm protected from the direct action of the rays provided a large enough portion of the worm were exposed, and particularly if the dose applied to the exposed portion were even greater than would be

TABLE 5

SUMMARY OF RESULTS FOLLOWING SECTION OF REGIONALLY IRRADIATED WHOLE ANIMALS (30-40 MM.) INTO APPROXIMATELY EQUAL SIXTHS AS INDICATED IN FIGURE 2

(—) Total or partial inhibition of regeneration, depending on the dose.
(H) Normal regeneration of head; (T) Normal regeneration of tail

Types of exposure	Number of experiments	Regeneration in respective regions					
		a	b	c	d	e	f
I	18	—	H-T	H-T	H-T	H-T	H
II	18	—	—	H-T	H-T	H-T	H
III	10	—	—	—	—	H-T	H
IV	15	—	—	—	—	—	H
V	10	T	H-T	H-T	H-T	H-T	—
VI	18	T	H-T	H-T	H-T	—	—
VII	18	T	H-T	H-T	—	—	—
VIII	12	T	—	—	—	—	—
IX	10	—	H-T	H-T	H-T	H-T	—
X	12	—	—	H-T	H-T	—	—
XI	10	T	—	—	—	—	H
XII	12	T	H-T	—	—	H-T	H
C	15	—	—	—	—	—	—
C′	10	T	H-T	H-T	H-T	H-T	H

required for a given degree of inhibition. This situation of continuity of the blood system and the coelomic fluid obtains in *Nais paraguayensis;* the septa are so perforated as to allow not only the interseptal passage of large amounts of the body fluid but also passage of numerous large coelomic-fluid cells suspended therein.

There are two additional facts which support this conclusion. In the first place, the inhibitory effect of the radiation, as observed externally, is apparent very shortly after exposure, that is to say, it is evident as soon after cutting (whether the cutting be done before or after irradiation) as the earliest manifestation of regeneration could be expected in normal specimens. Stone ('32) found that no "latent period" was required prior to cutting irradiated specimens of Tubifex. In addition, histological examination of specimens fixed within three-quarters of an hour after exposure reveals certain abnormalities in the neoblasts. The details of these changes need not be discussed at this time; the point is, that the appearance of these changes, so shortly after exposure, is what might reasonably be expected if the effect of the rays were direct.

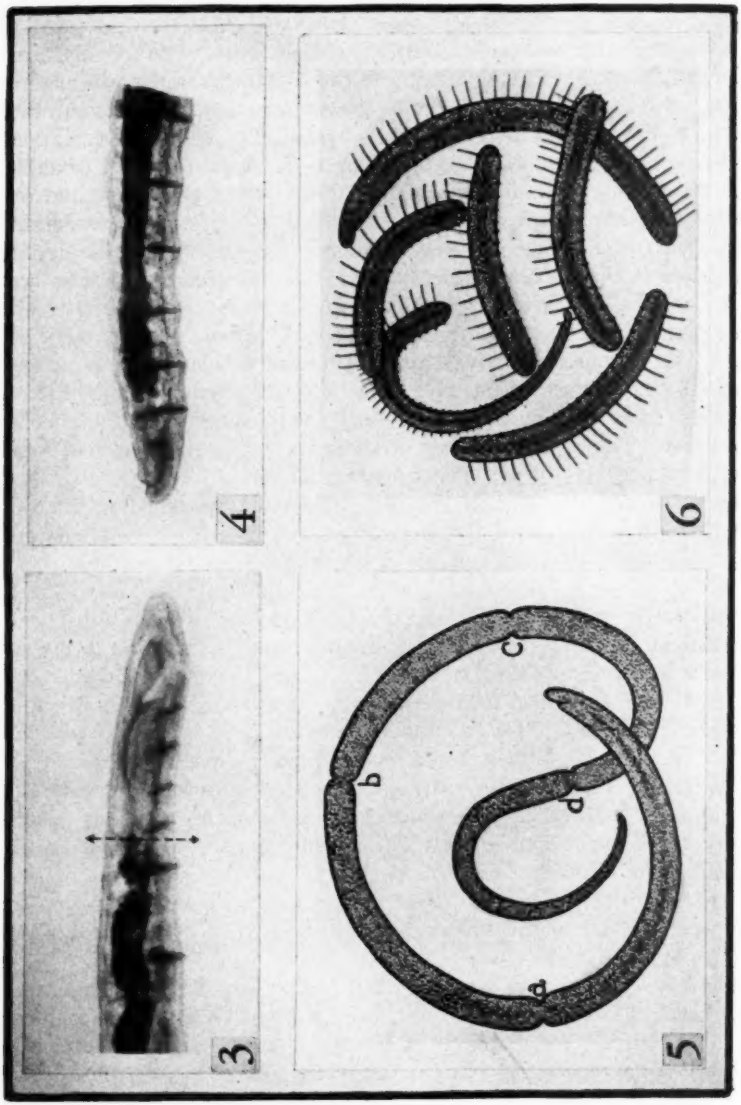
The foregoing experiments show furthermore that the cells effecting regeneration have their origin in loco, but they show this to be so only as opposed to any systemic origin of these elements, and in a not too precise way. That this particular problem is rather more subtle will be shown in the following section.

5. *Regional Irradiation of Partial Specimens*

As previously indicated, Butler's ('35) results with Amblystoma showed that the cells contributing to the limb regenerate must arise in loco, rather than being brought to the site of regeneration from other parts of the body. However, certain evidence as regards invertebrates warns against extending this conclusion to other groups. For example, certain regenerative elements of planarians (formative cells) and oligochaetes (neoblasts) have been described as migrating considerable distances to the region of regeneration. Thus, although the results obtained in the previous section show, in a somewhat less precise way than will be shown in this section, that the cells contributing to the regenerates have a local origin, as opposed, at least, to any systemic (e.g., haematogenic) derivation, the problem is, clearly, more subtle. And the experiments here have to do with an attempt to use this regional irradiation technique as an experimental approach to this specific question. The experiments are of an exploratory nature, and, as such, will be described in a very general and brief way, it being felt that they can serve only to indicate the possibilities of this technique in this regard. It seems clear, for several reasons, that *Nais paraguayensis* is by no means the most suitable of oligochaetes for the purpose.

On the basis of histological studies, the large neoblasts (mesodermal regenerative cells) of some oligochaetes have been described by some investigators as migrating only posteriorly and contributing to tail regeneration solely [Tubifex, Stone ('32, '33)], while in others their migration is described as both anterior and posterior [Lumbriculus, Turner ('34, '35)]. On the whole, the evidence is far from unequivocal. Histological studies (to be reported later) have revealed that the situation in *Nais paraguayensis* is rather similar to that described for Tubifex by Stone ('32, '33), wherein the neoblasts migrate only posteriorly and play no immediate rôle in head regeneration; the situation differs from Tubifex, however, in that the neoblasts are present as a persistent cell stock. These present experiments were undertaken after some knowledge of this histological picture in *Nais paraguayensis* had been obtained.

Briefly, a number of head and tail pieces, approximately one-third adult length, were ablated from mature worms; these were introduced into capillaries, irradiated over known distances, and subsequently observed for regeneration (*a*) immediately following the exposure and (*b*) later, following one or more ablations at points of varying relationship to the original irradiated region. By reason of the relative small-


4
6
3
5
c
b
d
a

FIGURES 3-6

FIGURE 3

Lateral view of a newly regenerated head, after about two days' growth; its posterior limit is indicated by the broken line. Photomicrograph of a living specimen.

FIGURE 4

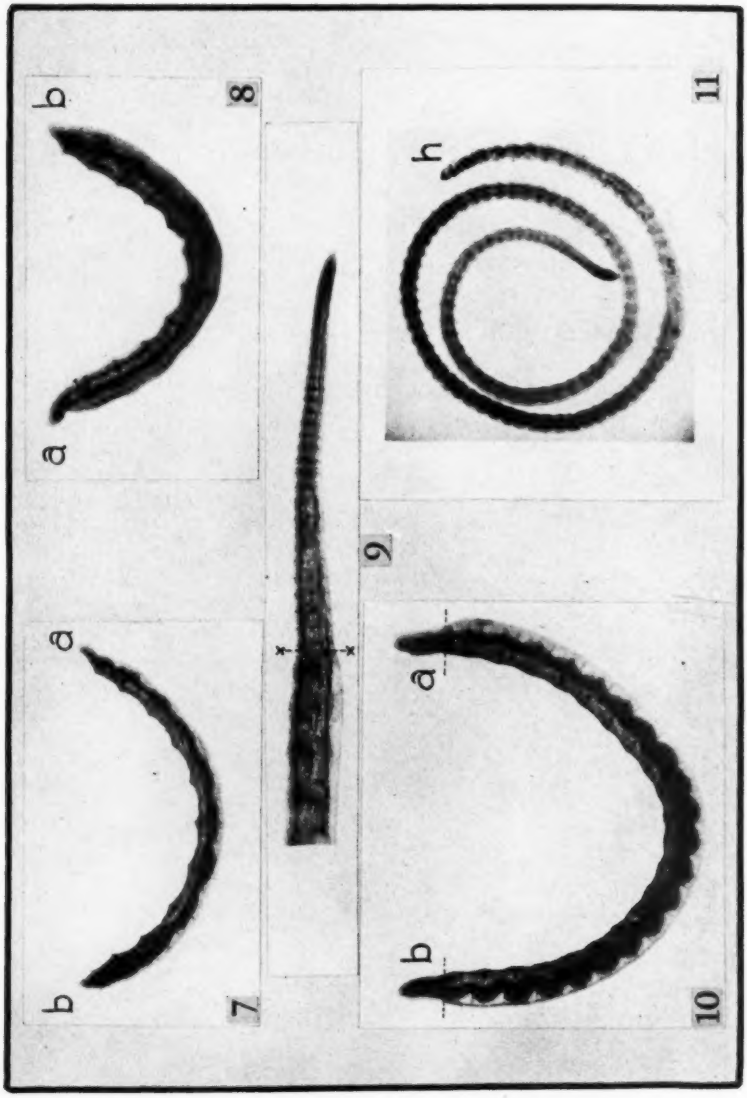
Lateral view of the posterior extremity of an adult specimen. Photomicrograph of a living specimen.

FIGURE 5

Drawing of an adult specimen just prior to its separation into five fragments; the loci of separation are indicated at *a*, *b*, *c* and *d*; setae omitted.

FIGURE 6

Drawing of six fragments immediately following the fragmentation of an adult specimen.


a
b
7
a
b
8
9
a
b
10
h
11

FIGURES 7-11

FIGURE 7

Cut fragment, seven days after irradiation (8,000 *r*); *a*, cephalic knob; *b*, anal knob.

FIGURE 8

Cut fragment, eleven days after irradiation (8,000 *r*); *a*, cephalic knob; *b*, anal knob.

FIGURE 9

Posterior regenerate of an unradiated, twenty-segment fragment, after five days; x....x marks the anterior limit of the regenerate.

FIGURE 10

Unradiated fragment from the trunk region, showing anterior (*a*) and posterior (*b*) regenerates about 40 hours after cutting; proximal limits of regenerates indicated by broken line.

FIGURE 11

Adult specimen of *Nais paraguayensis;* anterior extremity is indicated at (*h*).
(All figures are photomicrographs of fixed and stained specimens.)

ness of the distances involved, their exact ascertaining, under the experimental conditions prevailing, offers no small difficulty; nevertheless this was accomplished with reasonable accuracy. It may be added that the above difficulty appears to be the most likely explanation for the somewhat varied results obtained in this admittedly limited series of experiments.

The results, cumulatively, appear to add up as follows:

(*a*) Posterior regeneration may occur from an irradiated extremity, provided the extent of that irradiated portion be no more than a certain maximum distance, 1.0-1.5 mm., from the adjoining non-irradiated region; this is interpreted as indicating that the regeneration is brought about by the posterior migration of protected neoblasts, from the adjacent non-irradiated region, to the site of the cut. Some study of sections of this experimental material and the controls tends to support this interpretation.

(*b*) Head regeneration does not occur from an irradiated extremity even though the extent of this exposed portion be less, by approximately one-half, than the maximum extent of radiation, of an extremity, permitting tail regeneration; there is indeed reason to believe that even a lesser extent of radiation of such an extremity would be sufficient to prevent regeneration of a head. This would seem to provide experimental indication in support of the conclusion derived from histological studies, namely, that head regeneration is more strictly local, that neoblasts do not play the rôle here which they play posteriorly. It may also be added here that when an equally small extent of the anterior extremity of such tail pieces is protected, while the remainder is irradiated (approximately one-twelfth protected and eleven-twelfths irradiated), head regeneration ensues.

On the whole the results provide some experimental evidence in support of the concept that the cells effecting head and tail regeneration are essentially local in their origin, with reservation for that allowance which must be made for a limited posterior migration of neoblasts in the regeneration of the tail.

The few experiments just outlined may be compared to those of Zhinkin ('34). This worker grafted posterior somites of an irradiated Rynchelmis (Oligochaeta) (irradiated sufficiently to preclude posterior regeneration) to the cut posterior end of a non-irradiated worm; subsequently the neoblasts of the non-irradiated portion (presumably activated by the cutting) migrated posteriorly into the irradiated transplant, in which normal regeneration subsequently took place.

D. Discussion

The foregoing observations were made as being properly antecedent to a study of the complementary problem of the internal cellular phenomena attending normal regeneration and the inhibition thereof by x-rays. Several facts have been revealed, which, in the light of data on other invertebrates, suggest that with them might be correlated certain histological features. Clearly, a full discussion of these matters does not fall within the scope of the present report.

Two features have emerged from the study of the fragmentation process which would appear to warrant particular attention in the above regard: (*a*) Since it is known that in some oligochaetes there occurs an accumulation of certain regenerative cells at the site of the fission-zone, the occurrence of the loci of separation in *Nais paraguayensis* suggests the possibility of some comparable internal situation in these regions; (*b*) the fact that the head fragment is generally longest suggests that this might be associated with some peculiar distribution of regenerative elements.

Similarly, the information gained by the study of the externalities of regeneration has shown the relative importance of several factors in the process. For example, the fact that posterior regeneration will not take place from a head unless three trunk segments are left attached, challenges one to look for histological evidence with which this limitation might possibly be correlated. Again, the fact that, excepting in the extreme anterior and posterior regions, regeneration occurs with equal readiness at different levels of the body, and indeed in very small pieces, suggests among other things, a general distribution of neoblasts, that type of regenerative cell which has been shown, in other oligochaetes, to be so important in regeneration, and which Randolph ('92) has reported to be so particularly abundant in naids.

Having established that regeneration in *Nais paraguayensis* is inhibited by x-rays, it remains to be seen in how far the clue to this suppression may be found internally by comparison of the histology of irradiated and normal material. Also, the fact that there exists a differential in the ease with which anterior and posterior regeneration may thus be inhibited, suggests, on the basis of the generalization of Bergonié and Thribondeau ('06), that regeneration at the two extremities is accomplished, at least in part, by cells which possess, at the time of irradiation, different degrees of differentiation. There are other manifestations of irradiated material which, it would appear, may ultimately be correlated with certain internal cellular character-

istics of the normal animal. The demonstration that the loss of regenerative capacity does not preclude the ability to fragment, together with the fact that pieces of extremely small size can regenerate (pieces of such small size as are incapable of fragmenting), establishes the independence of these two phenomena.

The experimental proof that the effect of x-radiation in preventing regeneration in oligochaetes is a local and direct one, provides a basis for further studies, as indicated by the experiments reported here on the local nature of the regenerative process. Clearly, a knowledge of x-ray effects in terms of attendant histological and cytological changes is calculated to provide a better insight into the more fundamental aspects of regenerative processes.

E. Summary

1. By way of introductory observations, the process of fragmentation has been described in its gross normal features, and in relation to the size of the specimens and decapitation.

2. The regeneration of fragments has been studied in reference to: the gross morphology and chronology of restoration, regenerative capacity along the antero-posterior axis and in extremities, and the effect of size in regenerating pieces. Two types of regulatory phenomena have been described.

3. Sufficient exposure to x-rays inhibits regeneration; the inhibition is roughly proportional to the total-*r* applied; anterior inhibition requires a higher dose of radiation than does comparable posterior inhibition; several developmental manifestations of irradiated specimens have been described; the capacity to fragment is not precluded by the complete inability to regenerate; certain pertinent radiobiological data have been presented.

4. Regional irradiation experiments, a technique for which has been described, demonstrate that the inhibitory effect of the x-rays is local and direct. The cellular origin of the anterior and posterior regenerates is essentially local, as opposed to any systemic derivation; the reservations which must be made with reference to this last point have been indicated experimentally.

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THE GLYCOGEN CONTENT OF THE EMBRYO OF RANA PIPIENS DURING DEVELOPMENT

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As a preliminary to investigation on the action of inhibitors of carbohydrate metabolism in the Anuran embryo, it became desirable to know the glycogen content at each stage of development, and especially at those stages coming between fertilization and neurulation. Needham (1) has reviewed researches prior to 1931, and Waddington, Needham, and Brachet (2) have summarized subsequent investigations up to 1936. Since then Needham (3) has reviewed the field up to 1939. Among early researches, mentioned by Needham, those of Konopacki and Konopacka are of interest. These authors, using a histochemical staining method, demonstrated a loss of glycogen from the fertilized egg. This loss was partially restored by the time of gastrulation. Following gastrulation and neurulation they reported a decline in the glycogen content of the embryo which steadily continued until hatching. Other authors have also reported the post-neurulation fall in glycogen content of the amphibian egg (see Needham, 1). Among these are Brachet and Needham (4), who, although they confirm the post-gastrulation loss, deny the statements of Konopacki and Konopacka to the effect that there is a loss of glycogen before gastrulation. Brachet and Needham (4), however, have not reported estimations of glycogen on those stages succeeding fertilization and preceding gastrulation. Woerdemann (Waddington *et al.*, 2), Heatley (5), and Heatley and Lindahl (6) have demonstrated the sudden loss of glycogen from the dorsal lip of the gastrula during invagination of the dorsal ectoderm. In the present investigation, an effort has been made to obtain more detailed data on the changes in total glycogen of the developing Anuran embryo.

Eggs obtained from *Rana pipiens* females by the pituitary stimulation method (Rugh, 7) were artificially fertilized with sperm suspensions and allowed to develop in pond or tap water at room temperature. At various stages of development, eggs in the same stage were

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counted out under the binocular dissecting microscope in groups of 50. To standardize this procedure the timetables of Shumway (8) for the development of this species were consulted. These eggs were then fixed in hot 30 per cent KOH for glycogen precipitation by the method of Good, Kramer, and Somogyi (9). The glucose hydrolyzed from glycogen was estimated by the colorimetric method of Gibson (10). Glycogen is expressed (Table 1) in gammas per egg, which makes the

TABLE 1

GLYCOGEN CONTENT OF THE EMBRYO OF THE FROG, *Rana Pipiens*, DURING DEVELOPMENT

No. of stage*	Stage*	Hrs. dev.*	Avg. γ's glycogen/egg.	% Loss	No. expts.
1	Unfertilized	0.0	100.0	0.0	6
2	Gray- crescent	1.0	86.0	14.0	2
3	2-cell	3.5	81.0	19.0	4
4	4-cell	4.5	81.0	19.0	4
5	8-cell	5.7	83.5	16.5	4
6	16-cell	6.5	77.0	23.0	2
7	32-cell	7.5	73.0	27.0	4
8	Mid-cleavage	16.0	76.5	23.5	4
9	Late cleavage	21.0	69.0	31.0	2
10	Early gastrula	26.0	76.5	23.5	4
11	Mid-gastrula	34.0	87.5	12.5	4
11.5		38.0	98.0	2.0	6
12.25	Late gastrula	45.0	97.5	2.5	4
12.5		48.0	99.5	0.5	4
13.0	Neural plate	50.0	87.0	13.0	6
13.5		55.0	98.0	2.0	4
14.0	Neural folds	62.0	83.0	17.0	2
15.0	Rotation	67.0	87.5	12.5	4
16.0	Neural tube	72.0	78.0	22.0	2
17.0	Tail bud	84.0	72.5	27.5	4
18.0	Muscular response	96.0	62.0	38.0	2
19.0	Heart beat	118.0	63.5	36.5	6
19.5		130.0	56.5	43.5	4
20.0	Hatching	140.0	50.0	50.0	2
20.5		152.0	34.0	66.0	2
21.0	Mouth open	162.0	33.0	67.0	2
21.5		177.0	32.0	68.0	2

*Shumway (8).
(Undescribed stages interpolated from Shumway tables.)

results free from considerations of weight changes in the developing egg.

An examination of Table 1 will show that there is a marked loss of glycogen from the cleaving egg. By the time of mid-cleavage the glycogen has decreased from an average value of 100 to a value of 76.5, a loss of 23.5 per cent. This value rises again, reaching almost its original level at a stage just preceding neural plate formation.

There is no indication of an over-all loss of glycogen during gastrulation. This is in opposition to the report of Heatley and Lindahl (6) who found a decrease over the whole egg during gastrulation, although it was especially marked in the invaginating dorsal lip. The table also shows that following neurulation there is a fairly steady decrease of glycogen, until at hatching the over-all loss was 50 per cent. The figures in Table 1 for glycogen in the early gastrula, the neurula, and the hatching tadpole are in close agreement with those of Brachet and Needham (4), although the present results show a somewhat higher content for the unfertilized egg than do theirs.

The possibility of an increase in glycogen before gastrulation is of considerable interest in the light of recent investigations (Waddington *et al.*, 2) into the evocating power of glycogen implants in the amphibian egg, and in the light of the hypothesis of Waddington *et al.* (2) that the responsibility for neurulation may lie in a loose complex consisting of glycogen-protein-evocator. It would also be of great interest to know whether the post-fertilization loss of glycogen, which we have shown, is due to the breakdown of desmo- or lyo-glycogen, or of both. Of more importance, however, would be to know what effect inhibition of the pre-gastrulation resynthesis of glycogen would have on the process of neurulation. Durken (Waddington *et al.*, 2) has shown that irradiation of the dorsal lip with ultra violet rays will not inhibit invagination, but will inhibit neurulation. This effect is believed to be predominantly one on carbohydrate metabolism. The possibility of this is discussed by Waddington *et al.* (2). It is believed that the present results agree with the hypothesis that neurulation is dependent upon the action of a rather large accumulation of glycogen-protein-evocator, and that this complex is in part supplied by a pre-gastrulation synthesis of its carbohydrate component.

A series of provisional experiments was run on the effect of insulin on embryos at various stages. No differences in results were obtained as between plain *vs.* protamine-zinc insulin.

At concentrations of 0.05 per cent or stronger, the inner layer of jelly assumed a brownish color. This effect resembled those obtained in previous experiments using phloridzin. Insulin did not hasten the rate of development.

Eggs in mid-gastrula (Stage 11) were completely inhibited at 0.2 per cent with moderate cytolysis, at 0.1 per cent some reached the neurula (Stage 15), while at 0.05 per cent eggs developed to Stage 17. The maximal non-damaging dose was found to be at approximately

0.02 per cent. No significant difference was found in this value as a function of stage at which embryos were placed in the presence of insulin.

In another series of experiments groups of 20 eggs were placed in bowls containing 100cc. of 0.01 per cent insulin in distilled water. After nine hours exposure at room temperature, glycogen determinations were made on both insulin and untreated control embryos. Results on individuals in Stages 7, 11, 13, and 15 showed no difference in glycogen content as a result of exposure to insulin.

In addition to glycogen determinations, the oxygen consumption was measured using a modified Warburg respirometer (Pomerat and Zarrow, 11) and no significant difference was found between insulin-treated and control embryos.

Summary

The glycogen content of developing *Rana pipiens* embryos decreases during early cleavage and rises again, reaching its original value at neurulation. Following neurulation, glycogen decreases steadily until the time of hatching.

Insulin inhibits development at a concentration above 0.02 per cent and shows no acceleration of development, change in glycogen content, or in O_2 consumption when sub-lethal concentrations are used. It has not been possible, therefore, to affect the movement of glycogen stores by insulin.

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RADIOBIOLOGICAL STUDIES ON TISSUE CULTURES: I. THE IMMEDIATE EFFECT OF X-RAYS ON CELL OUTGROWTH IN CULTURES OF FIBROBLASTS

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It is known that irradiation of cell cultures with radium- or x-rays causes inhibition of the mitotic activity and appearance of abnormal mitotic figures. Despite the alterations in the mitotic process the growth rate of the irradiated cultures remains unchanged for some time. Depression of outgrowth generally becomes evident, however, after a period of 24-48 hrs. With very large doses of x-rays a restriction or even suspension of growth may occur immediately.

The action of x-rays on the mitotic process has been the subject of extensive studies. The irradiation effect on outgrowth of cells in vitro, is only known, however, in rough outlines. Quantitative investigation of the effect of x-rays on the growth rate of cell colonies proliferating in vitro, seemed, therefore, desirable and has been undertaken along lines followed in our investigations on the effect of radium on the growth of fibroblast cultures (Halberstaedter, L., & L. Doljanski, 1937).

A. Materials and Technique

1. *Culture Technique*

Our experiments were carried out on standardized cultures of chicken fibroblasts derived from heart of 7-day-old embryos. The cell colonies were cultivated in hanging drops according to the usual procedure, using a mixture of fowl-plasma and embryo extract as the medium. Growth curves of the cultures were constructed by the planimetric measurement of outline drawings of the surface area made every 24 hrs.

2. *Experimental Procedure*

To test the effect of x-rays, the culture before transferring in fresh medium was divided in two halves, one serving as the control and the other for irradiation. The irradiation was carried out immediately

after transferring. After irradiation controls and experimental cultures were returned to incubator. The growth rate of the control and irradiated cell colonies were recorded over a period of three days.

3. *Radiological Technique*

Irradiations were carried out using a demountable x-ray tube which worked at a tension of 35 KV mostly on currents of 2 and 20 MA. The tube had a copper-anticathode and a window of aluminum foil, 30 m. in thickness. Absorption analysis showed that the rays penetrating through the window foil and the 0.03 mm. thick mica-coverglass of the cultures were mainly copper K-rays. The x-ray intensity at the distance of the irradiated subject was about 10,500 *r*/min. for a tube-current of 2 MA, and about 85,000 *r*/min. for 20 MA.

B. Results

In the following experiments x-ray doses between 500 and 300,000 *r* units were employed. All cultures which received x-ray doses up to 75,000 *r*, became surrounded after incubation by a more or less broad continuous growth zone, which is planimetrically measurable. When the dose is increased the growth of the cultures became sparse and highly irregular or restricted to a few solitary cells projecting from the explant. Above 75,000 *r*, therefore, determination of the size of the growth area by planimetric measurement of the surface becomes impracticable. The effect of dose greater than 75,000 *r* was therefore expressed by determining the relative number of cultures falling into the following categories: (*a*) continuous growth area, (*b*) sparse, non-continuous growth area, (*c*) emigration of individual cells, and (*d*) no outgrowth.

The effect of irradiations with doses up to 75,000 *r* units on the outgrowth of fibroblast colonies in vitro might be summarized as follows. A dose of 500 *r* produces no visible effect, and the irradiated cultures do not differ from controls. At 1000 *r* a slight inhibition of outgrowth appears to occur, but differences observed are within the range of error of the method. Cultures which received 2500 *r* show a small but distinct restriction of cell growth on the second and third day of incubation. There is a latency of 24 hrs. before the effect becomes manifest.

At 5000 *r* definite and marked restriction of cell outwandering was regularly observed. On the 3rd day of incubation the quotient of the growth areas of irradiated cultures to controls averaged 0.58. The

difference in growth rate between irradiated and non-irradiated cultures became evident in most cases on the second day after irradiation. On the first day the effect of x-rays was hardly appreciable.

The effect on cell outgrowth of doses of 10,000 and 25,000 *r* largely resembles that of 5000 *r*, but is somewhat more pronounced and already well measurable in most of the cultures on the first day. At 50,000 and 75,000 *r* the effect of irradiations is considerable and in all cultures quite evident after 24 hrs. The quotient of growth areas of irradiated cultures to controls, determined on the third day after irradiation is 0.26 at 50,000 *r* and 0.19 at 75;000 *r*.

The typical responses of cultures to varying doses of x-rays are plotted in Figure 1. The curves given were selected from sets of 15-17

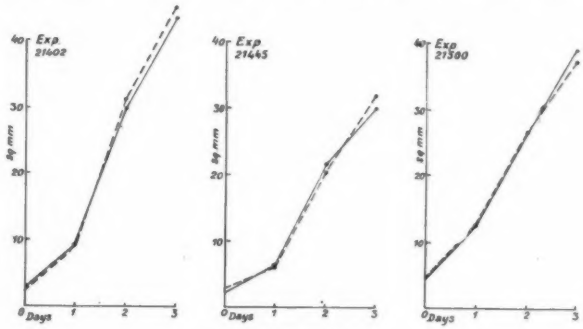

FIGURE 1*a*

IRRADIATED WITH 500 *r*

---------- Controls

———————— Irradiated cultures

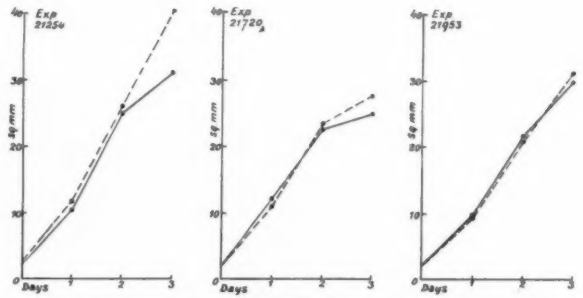

FIGURE 1*b*

IRRADIATED WITH 1000 *r*

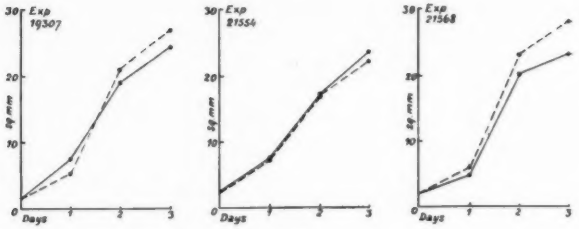

FIGURE 1*c*
IRRADIATED WITH 2500 *r*

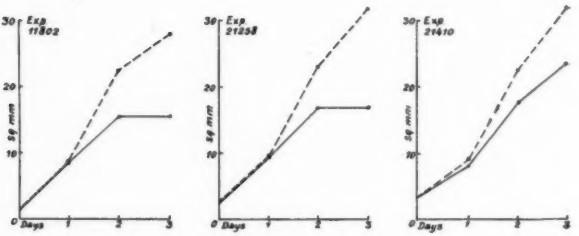

FIGURE 1*d*
IRRADIATED WITH 5000 *r*

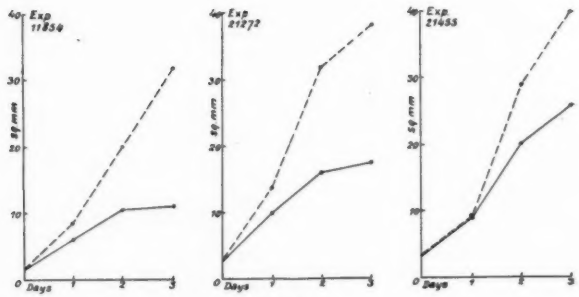

FIGURE 1*e*
IRRADIATED WITH 10,000 *r*

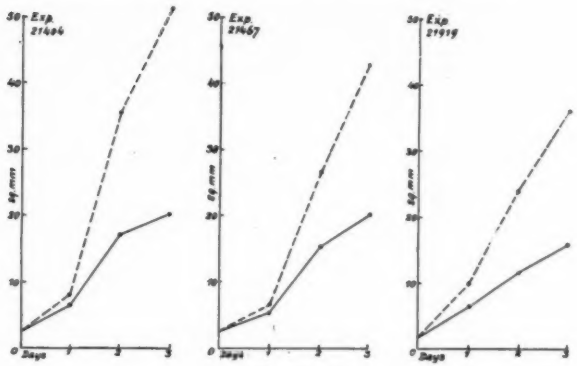

FIGURE 1*f*

IRRADIATED WITH 25,000 *r*

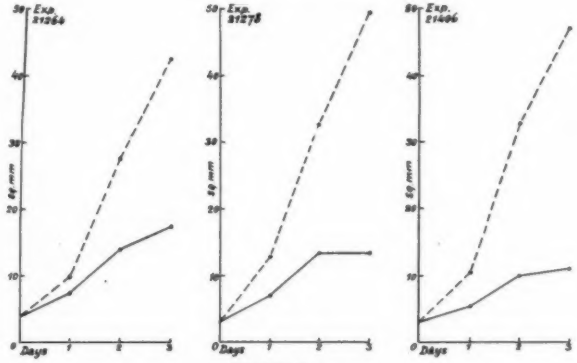

FIGURE 1*g*

IRRADIATED WITH 50,000 *r*

experiments performed for each x-ray dose. The complete quantitative data of all experiments are represented in Tables 1-8.

At 100,000 *r* appreciable outgrowth of cells from irradiated fragments still continues. After 24 hrs. the irradiated cultures become surrounded by loosely connected cells. This zone of growth does not increase on further incubation and the cells tend subsequently to separate and undergo a progressive degeneration. With still larger doses the growth of the cultures becomes more and more restricted, and the number of cell colonies which show only individual cell outgrowth becomes progressively greater.

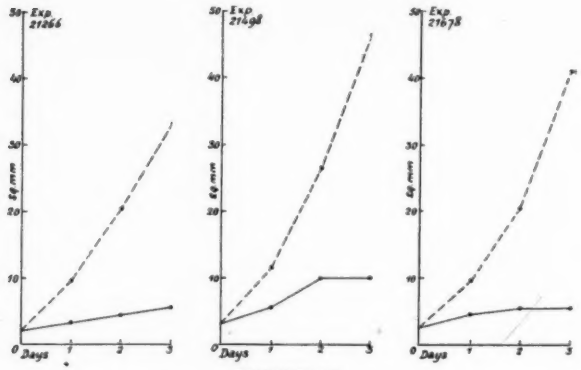

FIGURE 1*h*

IRRADIATED WITH 75,000 *r*

TABLE 1

IRRADIATION WITH 500 *r* UNITS

Experiment No.	Size (m/m^2) of growth area after 24 hrs. Exp.	Contr.	Exp. Contr. %	48 hrs. Exp.	Contr.	Exp. Contr. %	72 hrs. Exp.	Contr.	Exp. Contr. %
11785	2.4	1.7	141	11.0	11.7	94	19.9	19.6	102
11786	1.9	2.2	86	10.0	9.0	111	23.3	23.3	100
18909	3.6	3.7	97	11.8	10.5	112	20.5	22.5	91
18911	3.8	2.8	136	13.8	10.6	130	22.8	19.3	118
21402	6.4	6.2	104	26.9	28.7	94	40.2	42.7	94
21445	4.3	3.4	126	19.0	17.9	106	27.8	29.2	95
21500	7.7	7.7	100	21.7	21.9	99	34.0	33.2	103
21504	8.1	7.1	114	17.8	17.5	102	22.6	20.7	109
21506	5.9	5.3	111	19.5	17.6	111	27.4	25.0	109
21510	6.2	5.8	107	21.4	18.8	114	37.1	27.3	136
21654	4.7	4.7	100	16.0	14.7	109	18.8	16.7	113
21712	3.8	4.3	88	15.3	16.0	96	19.1	22.3	86
21718	5.4	4.8	112	17.8	17.3	103	24.2	22.1	110
21396	5.5	5.4	102	23.6	27.6	85	33.1	37.4	88
11790	1.3	1.2	108	9.8	8.6	114	17.3	22.6	77
Mean			109			105			103
Stand. error of the mean			±4.1			±2.9			±3.8

From doses of 100,000 *r* or more, moreover, cultures with no cell outgrowth at all appear. The proportion of cultures without signs of cell migration increases with the dose, appr. 30 per cent at 120,000 *r*, 53 per cent at 180,000 *r*, 63 per cent at 200,000 *r*, and reaching

TABLE 2

IRRADIATION WITH 1000 *r* UNITS

Experiment No.	Size (m/m^2) of growth area after 24 hrs. Exp.	Contr.	Exp. Contr. %	48 hrs. Exp.	Contr.	Exp. Contr. %	72 hrs. Exp.	Contr.	Exp. Contr. %
11797	7.7	8.9	87	22.8	25.8	88	26.6	28.0	95
11800	5.5	3.4	162	21.3	15.3	140	24.8	26.6	93
16571	3.7	3.4	109	14.1	12.8	110	18.2	17.3	105
18146	4.5	3.6	125	22.8	18.9	121	27.4	26.7	103
18150	3.1	4.3	72	15.5	18.7	83	20.4	20.7	99
18903	2.8	3.0	93	10.8	12.6	86	16.6	17.0	98
18905	3.0	4.0	75	10.5	10.8	97	17.5	17.5	100
21254	8.7	8.9	98	22.2	23.2	96	28.1	37.7	75
21720	4.6	4.1	112	20.2	21.1	96	22.7	25.7	87
21730	5.1	5.0	98	13.3	15.6	85	18.0	18.1	100
21909	8.1	8.6	94	27.3	23.6	116	31.2	27.0	116
21913	12.5	8.1	155	23.3	22.3	104	38.7	35.8	108
21951	6.4	7.7	83	16.4	16.4	100	22.7	27.2	83
21953	7.3	7.6	96	18.7	19.2	97	27.2	29.4	93
22037	3.0	2.3	130	12.9	19.5	66	17.4	22.2	78
22039	2.9	3.6	81	14.5	13.2	110	18.8	18.2	103
Mean			104			100			96
Stand. error of the mean			±6.6			±4.4			±2.7

TABLE 3

IRRADIATION WITH 2500 *r* UNITS

Experiment No.	Size (m/m^2) of growth area after 24 hrs. Exp.	Contr.	Exp. Contr. %	48 hrs. Exp.	Contr.	Exp. Contr. %	72 hrs. Exp.	Contr.	Exp. Contr. %
19254	2.6	3.3	79	12.6	14.0	90	20.0	21.8	93
19307	6.0	4.1	146	17.6	19.7	89	23.0	25.7	89
19311	6.1	7.6	80	12.7	18.7	68	14.1	22.1	64
19423	6.2	6.1	102	14.7	17.9	82	19.2	22.0	87
19513	3.2	3.0	107	9.8	11.9	82	18.1	19.2	94
19515	3.2	3.2	100	10.5	13.6	77	17.2	18.5	93
19527	3.2	4.4	73	11.5	13.7	84	20.6	20.5	100
21256	4.6	3.5	131	15.2	12.1	125	22.3	20.6	108
21554	4.6	5.2	88	14.2	14.8	96	20.6	20.0	103
21556	4.7	5.2	90	13.0	17.1	76	18.4	20.7	89
21562	3.1	4.4	71	12.9	16.4	79	18.9	21.1	89
21566	3.4	3.9	87	13.4	14.8	90	17.7	20.6	86
21568	2.8	3.8	74	17.5	20.7	85	20.5	26.6	77
21955	6.2	6.6	94	13.6	15.5	88	17.5	18.0	97
22072	3.9	4.2	93	11.9	14.9	88	18.5	18.8	98
Mean			94			87			91
Stand. error of the mean			±5.5			±3.4			±2.8

TABLE 4

IRRADIATION WITH 5000 *r* UNITS

Experiment No.	Size (m/m^2) of growth area after 24 hrs. Exp.	Contr.	Exp. Contr. %	48 hrs. Exp.	Contr.	Exp. Contr. %	72 hrs. Exp.	Contr.	Exp. Contr. %
11801	4.8	6.7	72	17.1	25.9	66	24.2	39.3	61
11802	7.6	6.8	112	14.6	20.7	70	14.3	26.3	55
11803	8.5	9.6	88	10.9	17.1	64	11.1	19.5	57
11842	5.7	5.7	100	8.7	15.1	58	11.9	29.3	41
11843	6.0	8.4	71	9.9	19.0	52	18.9	38.2	50
11844	4.3	5.7	76	9.8	11.2	87	12.8	22.1	58
11858	5.3	5.8	91	15.3	23.8	64	15.7	34.4	46
11859	4.8	4.8	100	13.4	19.3	69	13.3	25.5	52
19657	6.8	6.4	106	11.5	16.8	69	15.5	21.9	71
19659	6.2	6.6	94	14.5	21.3	68	19.3	26.3	73
19661	5.8	5.8	100	11.4	17.1	67	15.9	24.5	65
19663	5.3	5.7	93	13.6	18.6	73	16.8	25.5	66
21258	7.0	6.8	103	14.6	20.3	72	14.4	29.3	49
21270	8.4	11.1	76	14.6	23.5	62	18.5	30.6	60
21410	4.9	5.5	89	14.3	24.1	59	19.9	28.5	70
Mean			91			67			58
Stand. error of the mean			±3.6			±2.1			±2.5

TABLE 5

IRRADIATION WITH 10,000 *r* UNITS

Experiment No.	Size (m/m^2) of growth area after 24 hrs. Exp.	Contr.	Exp. Contr. %	48 hrs. Exp.	Contr.	Exp. Contr. %	72 hrs. Exp.	Contr.	Exp. Contr. %
11816	4.9	7.8	63	12.5	20.9	60	13.7	30.1	46
11821	2.2	5.6	39	5.0	16.2	31	4.8	18.6	26
11854	4.5	7.1	63	9.5	17.8	53	9.8	30.3	32
11857	7.0	12.3	57	10.9	21.3	51	14.6	40.9	36
11862	4.5	6.0	75	10.0	21.8	46	16.1	28.3	57
19742	2.8	2.9	97	11.1	19.3	57	17.5	28.8	61
19744	3.4	3.7	92	9.7	15.7	62	13.9	26.6	52
19748	4.7	5.8	81	12.5	19.4	64	19.8	32.6	61
21260	4.4	8.7	51	11.7	22.1	53	15.4	27.9	52
21272	7.9	10.9	72	14.0	28.0	50	15.5	35.0	44
21455	5.3	5.5	96	16.6	25.5	65	22.3	36.5	61
21578	3.1	3.2	97	10.8	18.8	57	12.7	23.6	54
21580	3.6	4.8	75	14.7	19.2	76	16.5	23.8	69
21714	5.9	5.6	105	15.3	16.2	94	17.3	19.7	88
21718	6.3	4.5	140	19.0	21.6	88	19.9	26.3	76
Mean			80			60			54
Stand. error of the mean			±6.5			±4.1			±4.2

TABLE 6

IRRADIATION WITH 25,000 *r* UNITS

Experiment No.	Size (m/m^2) of growth area after 24 hrs. Exp.	24 hrs. Contr.	24 hrs. Exp. Contr. %	48 hrs. Exp.	48 hrs. Contr.	48 hrs. Exp. Contr. %	72 hrs. Exp.	72 hrs. Contr.	72 hrs. Exp. Contr. %
11875	1.8	2.2	82	10.2	16.5	62	13.1	21.5	61
11878	2.3	3.2	72	11.3	18.5	61	15.2	25.3	60
16661	3.7	4.3	86	10.6	15.7	68	10.1	22.4	45
16665	3.0	3.2	94	7.6	12.0	63	8.9	19.0	47
18408	1.4	2.6	54	6.6	15.7	42	6.6	24.3	27
18410	5.0	5.8	86	12.0	18.8	64	15.7	24.9	63
18412	2.3	7.4	31	8.6	16.7	51	9.4	23.9	39
18414	2.4	5.4	44	6.8	13.9	49	8.8	23.6	37
21274	6.1	7.9	77	11.0	19.3	57	14.8	25.3	59
21404	4.1	5.2	79	14.7	32.7	45	18.2	48.9	37
21449	2.8	4.9	57	10.8	19.9	54	14.6	34.0	43
21453	2.2	6.6	33	10.3	16.0	64	13.4	28.0	48
21467	3.0	4.1	73	12.9	24.0	54	18.0	40.3	45
21514	5.2	7.0	74	11.4	22.0	52	12.4	35.7	35
21919	4.5	8.4	54	10.0	22.8	44	14.1	34.4	41
Mean			66			55			46
Stand. error of the mean			±5.1			±2.1			±2.8

TABLE 7

IRRADIATION WITH 50,000 *r* UNITS

Experiment No.	Size (m/m^2) of growth area after 24 hrs. Exp.	24 hrs. Contr.	24 hrs. Exp. Contr. %	48 hrs. Exp.	48 hrs. Contr.	48 hrs. Exp. Contr. %	72 hrs. Exp.	72 hrs. Contr.	72 hrs. Exp. Contr. %
11879	3.8	4.4	86	6.1	29.1	21	6.3	36.4	17
11880	3.1	6.2	50	9.0	18.2	50	10.0	32.2	31
11881	5.8	6.2	94	15.4	30.9	50	15.8	43.2	37
11883	4.4	8.2	54	6.8	18.0	38	6.6	20.3	32
11884	4.8	10.5	46	6.2	23.4	27	7.0	29.7	24
11885	2.9	9.5	30	6.1	21.0	29	6.6	29.2	23
18416	2.4	3.8	63	4.6	14.0	33	7.8	22.4	35
18422	2.0	3.7	54	4.7	11.2	42	6.0	21.8	28
21264	3.7	7.3	51	10.2	24.8	41	13.6	40.0	34
21278	4.1	9.8	41	10.4	29.8	35	10.0	47.0	21
21400	5.9	5.1	116	6.9	29.8	23	6.9	36.8	19
21406	2.5	7.6	33	7.4	29.8	25	8.3	45.0	18
21451	2.7	5.5	49	6.7	23.9	28	7.2	38.5	19
21459	2.4	6.5	37	5.0	21.7	23	6.4	41.7	15
21544	1.8	2.9	62	6.0	10.1	59	6.8	20.3	34
21550	3.6	4.8	75	6.6	15.8	42	8.9	28.7	31
21558	2.0	5.7	35	7.6	14.3	53	7.4	25.4	29
Mean			57			36			26
Stand. error of the means			±5.5			±2.8			±1.7

TABLE 8

IRRADIATION WITH 75,000 *r* UNITS

Experiment No.	Size (m/m^2) of growth area after 24 hrs. Exp.	Contr.	Exp. Contr. %	48 hrs. Exp.	Contr.	Exp. Contr. %	72 hrs. Exp.	Contr.	Exp. Con.r. %
11888	0.5	9.1	5	0.5	20.8	2	0.5	34.2	1
11889	0.6	4.2	14	0.6	17.7	3	0.6	18.8	3
11892	1.1	5.2	21	1.2	14.7	8	1.2	15.4	8
21266	1.1	7.3	15	2.4	18.0	12	3.3	31.2	11
21498	2.8	8.5	33	7.9	23.7	33	6.4	43.5	15
21508	2.9	5.8	50	4.8	15.3	31	5.1	26.9	19
21512	3.2	6.7	48	7.0	16.8	42	7.9	22.0	36
21520	3.5	6.1	57	6.8	15.0	45	7.8	26.0	30
21552	3.5	5.9	59	5.7	27.9	20	11.1	44.0	25
21664	2.4	5.4	44	5.7	15.8	36	6.8	18.8	36
21668	2.9	5.8	50	4.6	13.4	34	5.6	21.6	26
21670	1.7	5.3	32	3.1	15.9	20	2.9	25.4	11
21678	1.6	6.6	24	4.1	17.5	23	3.7	38.1	10
21686	1.8	4.3	42	5.3	14.4	37	5.9	20.0	30
21925	5.2	7.1	73	7.4	15.2	49	5.2	28.5	18
Mean			38			26			19
Stand. error of the means			±5			±3.9			±3

100 per cent at 260,000 *r*. We designate the latter dose as the "immediately lethal."

The determination of the dose range within which an immediately lethal effect may occur, is based on experiments with a total of 452 cultures. The complete results of these experiments are presented in Table 9.

TABLE 9

Doses in 1000 *r*	Total of irradiat. cultures	Effect of irradiation* *a*	*b*	*c*	*d*
100	40	18	13	3	6
120	42	14	10	6	12
140	41	6	6	9	20
160	40	7	4	8	21
180	47	5	6	11	25
200	40	1	6	8	25
220	40	1	3	5	31
240	40	—	—	4	36
260	41	—	—	—	41
280	40	—	—	—	40
300	41	—	—	—	41

**a*) continuous growth area;
b) sparse, non-continuous growth area;
c) emigration of individual cells;
d) no outgrowth.

C. Discussion

The great resistance of cell cultures to x-rays has long been known. Krontovski ('26), Schubert ('27), and Roffo ('28) failed to influence the growth of cell colonies by x-rays, although relatively large doses were used by them. Laser and Halberstaedter ('29) showed subsequently that this insusceptibility was only apparent, the effect of irradiation on cell outgrowth being delayed. Cultures irradiated with 1000 and 10,000 *r* grow as well as the controls for some time after irradiation but manifest a definite restriction of the growth rate after a latent period of 24-48 hrs.

The delayed x-ray effect on growth of tissue culture was also demonstrated with a different technique by Fischer and Baastrup ('30). These authors irradiated the culture of fibroblast with an x-ray dose of 20 Sabourand units. They found no inhibition of growth in the irradiated cultures themselves, but a definite retardation of the growth rate after transferring the irradiated cultures into fresh medium. These findings were extended later by Santesson ('28) and by Cox ('31) for x-rays, and by Spear ('30) for radium.

Immediate inhibition and complete arrest of growth (immediate lethal effect) in cell cultures was demonstrated by Doljanski, Trillat, Lecomte du Nouy, and Rogozinski ('31), using very high doses of x-rays. To suppress all cell migration in fibroblast cultures an x-ray dose of 120,000 *r* was required. According to Goldfeder ('38) explants of embryonic chicken hearts fail to show all cell outgrowth after irradiation with 30,000 *r*. In a later investigation using different technique, Goldfeder ('40) found that cell outwandering is only suppressed by doses in the range of 50,000 to 260,000 *r*, the x-ray susceptibility of explants varying from organ to organ. Chambers and Cameron ('41) investigated the effect of x-rays on explants of kidney and found suppression of fibroblast outgrowth at 24,000 *r*; wandering cells were found less sensitive, epithelial cells more so.

The only systematic investigation of the retarding effect of x-rays on the growth rate of cell cultures in vitro is due to Faber ('35). This author showed that doses of 100-200 *r* produce a very slight effect on the outgrowth of fibroblast cultures. With increase in dose the inhibitory effect becomes proportionally greater. At 1200 *r* the inhibition attained was maximal, and was not increased by 4-5 fold augmentation of the dose applied.

In the present experiments which were devoted to quantitative study of the growth inhibitory effect of x-rays on fibroblast cultures in the

3-day period over which observations were carried out, the following results were obtained:

(*a*) Doses of 500 to 1000 *r* produced no measurable effect on outgrowth of fibroblast in vitro.

(*b*) Inhibition of growth becomes only discernible at 2500 *r*.

(*c*) At 5000 *r* a marked inhibition of growth is produced. Generally, however, this effect becomes apparent only after a latent period of 24 hrs.

(*d*) The effect on cell outgrowth of doses of 10,000 and 25,000 *r* resembles that of 5000 *r*, but is somewhat more pronounced and already well measurable in most of the cultures on the first day of cultivation.

(*e*) At 50,000 and 75,000 *r* the effect of irradiation is considerable and evident without exception already 24 hrs. after irradiation.

(*f*) At doses of 100,000 *r* and higher the growth of the culture becomes more and more restricted and the number of cell colonies which show only solitary cell outgrowth, progressively rises.

(*g*) At doses of 100,000 *r* irradiated cultures with no cell outgrowth at all are regularly encountered. The percentage of irradiated cultures without sign of cell migration increases with rising doses.

(*h*) At 260,000 *r* complete arrest of outgrowth in all cultures irradiated is attained ("immediate lethal effect").

The response of the culture to irradiation with increasing doses of x-rays is graphically represented in Figures 2 and 3. The curve in Figure 2 gives the percentage of irradiated cultures which showed no sign of migration ("immediate lethal effect") as a function of the x-ray dose.

The progressive restriction of outgrowth in irradiated cultures with increase in dose is represented in Figure 3. The curve falls steeply up to 5000 *r* and beyond this point flattens out and declines gradually to the endpoint.

In interpreting the shape of this curve it must be kept in mind that the increase in the growth area of the cultures is dependent on two cell functions—proliferation and outwandering. Each of these functions has a different x-ray susceptibility. Cell proliferation is altered at very low doses (Spear, '35) and irreparably damaged at 5000 *r*.[1] Doses of this order affect outwandering of the cells only slightly if at all. Fifty-fold greater doses than irreparably injure the mitotic process

[1]Unpublished experiments.

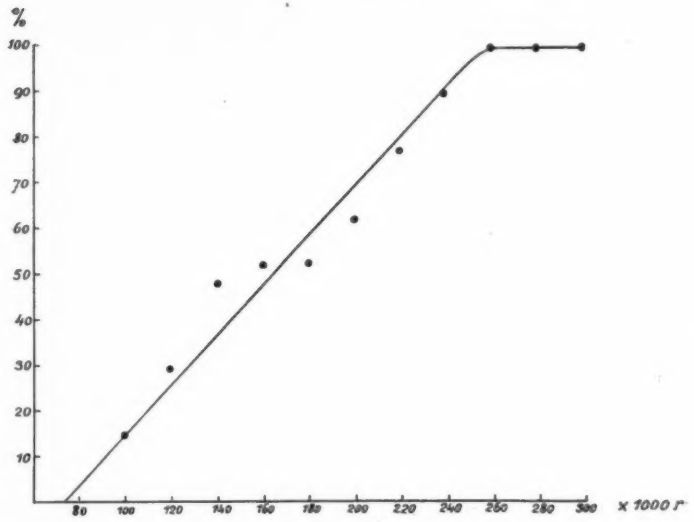

FIGURE 2

PERCENTAGE OF IRRADIATED CULTURES SHOWING IMMEDIATED LETHAL EFFECT AS A FUNCTION OF X-RAY DOSE APPLIED

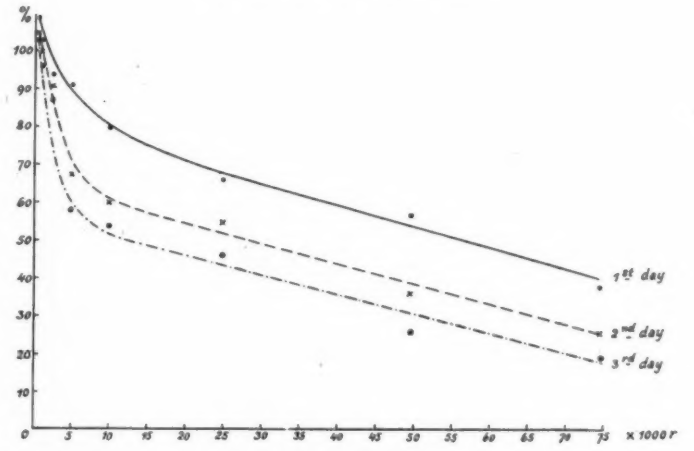

FIGURE 3

EFFECT OF X-RAYS ON OUTGROWTH OF FIBROBLAST CULTURES

Abscissae—x-ray dose in *r* units.

Ordinates—the relation in percentage of the size of the growth area in irradiated cultures to that of the controls after 1, 2, and 3 days of incubation.

are necessary for full arrest of cell migration. The circumstance that the growth of cultures is conditioned by two factors—one high susceptible to the x-rays and the other relatively resistant—suggests a plausible interpretation of the course of our curves. They may be regarded as composed of two parts. The first part which slopes steeply with increasing dose up to 5000 *r*, reflects the alteration of the mitotic activity. The succeeding flat part of the curve is probably a resultant of the summative effect of radiation on the mitotic function and on cell motility. The latter declines gradually with increasing dose and is finally inhibited completely.

The dose of 260,000 *r* here found immediately lethal for cultures of fibroblasts is twice the dose previously found lethal for the same type of cell by Doljanski, Trillat, Lecomte du Nouy, and Rogozinski ('31). This difference is due to the fact that in the present study the term "lethal dose" was taken to mean complete absence of all outgrowth, even of solitary cells. The lethal dose for chicken fibroblasts, as here established, is slightly greater than that for normal rat fibroblasts as determined by us in a previous investigation (Halberstaedter, Goldhaber and Doljanski, '42), using a similar technique.

D. Summary

The immediate growth-inhibiting effect of x-rays on fibroblast cultures in the dose range of 500 to 300,000 *r* has been investigated.

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RELATIVE GROWTH OF THE EUROPEAN EDIBLE CRAB, *CANCER PAGURUS*: I. GROWTH OF THE CARAPACE

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A. INTRODUCTION

This is the first of several papers dealing with the growth and form of the European Edible Crab, *Cancer pagurus*. The present paper is the outcome of an investigation carried out in Great Britain during the year 1934-35 which was made possible for the author by the granting of a Royal Society of Canada Fellowship in Zoölogy for that year. Most of the work was carried out through the aid of the excellent facilities of the Laboratory of the Marine Biological Association of the United Kingdom at Plymouth.

In addition to the Royal Society of Canada the writer is indebted to Dr. E. J. Allan who was then director of the Marine Biological Laboratory at Plymouth; to Professor Julian S. Huxley, then Professor of Zoölogy at King's College, University of London, and later Secretary of the Zoölogical Society of London, for valuable help and advice throughout the entire investigation, and to Professor R. A. Fisher of University College, University of London, for advice in connection with statistical procedures.

A copy of the complete data, of which this is a part, is on file with the Royal Society of Canada.

B. THE EUROPEAN EDIBLE CRAB

Cancer pagurus, the European Edible Crab, is almost certainly the largest species of the economically important genus *Cancer*, a genus that has a world-wide distribution and that is used commercially in a number of countries. Individuals of *Cancer pagurus* sometimes attain a weight of 10-12 pounds and a carapace width of 28 cm. The species is found in the United Kingdom and northwestern Europe where it inhabits rocky shores.

FIGURE 1
Cancer pagurus, ADULT ♂, DORSAL VIEW

1. *Relative Growth*

The *Cancer pagurus* data, probably the most extensive collected for any species of animal up to the time of the investigation (1934-35), are herein analyzed by the method of Huxley (Huxley, 1932). This method is too well known to require more than a brief explanation here.

Relative growth is ordinarily described in terms of "*k*." The nature of this constant may be seen from the following formula. If the plot may be fitted by a straight line the slope-intercept formula for this will be:

$$\log p = \log b + k \log w$$

where p represents the part or dimension considered and where w represents the whole (here the carapace length) and b and k are constants. Transforming, this becomes:

$$p = b\, w^k$$

The constant k, which represents the ratio between the growth of the part and that of the whole, is of considerable interest. In dealing with animals of unknown age, as is here the case, it introduces a dynamic approach to static material.

2. *How the Cancer pagurus Data Were Collected*

All the measurements used in the present study were made by the writer during the year 1934-35. The crabs were collected in the vicinity of Plymouth, England. Many collections were made on the shores of Drake Island, a small fortified island named after the famous navigator and situated in Plymouth harbor. In order to include the larger sizes, arrangements were made to measure a large number of crabs brought in by the fishermen in addition to those caught by the author. The commercial animals were used only when they could be measured before being cooked since cooking results in a certain amount of shrinkage.

Two sources of error have been eliminated in this study since the measurements were all made by one person and all the animals were collected in one locality.

All the measurements were made with the aid of a Glogau sliding jaw vernier caliper and were recorded to the nearest hundredth of a centimeter. It has been found that the rigidity of the crab exoskeleton is conducive to great accuracy of measurement. The following are the carapace dimensions that were studied (see also Figure 2):

1. The maximum length of the carapace.
2. Carapace width *A* (The interorbital width; the distance between the outer margins of the eye orbits).
3. Carapace width *B* (The carapace width at the second lateral notch).
4. Carapace width *C* (The carapace width at the fourth lateral notch).
5. Carapace width *D* (The carapace width at the sixth lateral notch).
6. Carapace width *E* (The carapace width at the widest point).
7. Carapace width *F* (The carapace width at the ninth lateral notch).
8. Carapace width *G* (The carapace width at the tenth lateral notch).
9. Carapace width *H* (The carapace width near the posterior margin; position marked by a slight ridge).

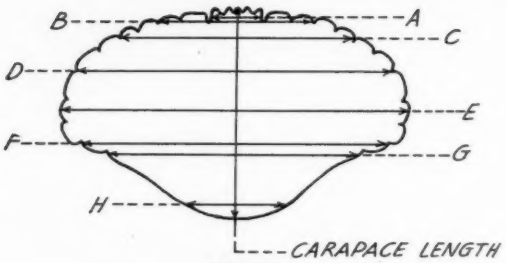

FIGURE 2
DIAGRAM OF CARAPACE TO SHOW DIMENSIONS STUDIED

3. *Relative Growth of the Carapace*

The foregoing chart (Figure 2) graphically illustrates the carapace measurements that were made. It will be noted (Table 1) that the data have been grouped into logarithmic size-classes. This is believed to have been a new departure at the time when it was carried out (1934) and before actually grouping the measurements in this manner the writer discussed the matter with Professor R. A. Fisher who signified his approval. The data were grouped in this manner principally because the writer wished to avoid the grouping of points on a log-log chart which results from the use of equal size classes, e.g., 1.0-1.9, 2.0-2.9, 3.0-3.9, 4.0-4.9, etc. The points, as grouped in the present work, should be approximately equally spaced on a double logarithmic chart (see Table 1 and Figure 3). The advantages are obvious.

Throughout the study an attempt was made to keep the number of measurements for any given part as nearly equal for the two sexes as possible. For the various carapace dimensions listed above, 5246 measurements were made, 2615 for the females and 2631 for the males. Figure 3 illustrates some of the plots obtained from these data. The results of plotting all of the carapace measurements are summarized in Table 2.

It will be noted that the values for k range from 0.70 to 1.12, a rather narrow range. Weymouth and MacKay (1934 and 1936) have shown that another member of the genus, *Cancer magister*, is likewise characterized by extreme conservatism in all aspects of relative growth.

4. *Carapace Gradients*

The values of k given in Table 2 are graphically shown in Figure 3. It will be observed that the values for k with one exception are not

TABLE 1

MEANS OF CARAPACE WIDTHS *A* AND *B* TOGETHER WITH LENGTHS OF CARAPACE OF MALE AND FEMALE CRABS

Lower limits of size classes	Males No.	Males Mean carapace length	Males Mean width dimension *A*	Females No.	Females Mean carapace length	Females Mean width dimension *A*
0.00	6	0.87	0.55	8	0.83	0.53
1.00	11	1.08	0.65	9	1.10	0.65
1.26	19	1.44	0.82	21	1.45	0.81
1.58	31	1.72	0.95	36	1.81	0.96
2.00	33	2.24	1.13	41	2.25	1.14
2.51	34	2.86	1.35	48	2.84	1.35
3.16	36	3.57	1.56	57	3.51	1.57
3.98	13	4.43	1.86	24	4.39	1.85
5.01	11	5.79	2.25	12	5.46	2.20
6.31	64	7.39	2.70	52	7.08	2.70
7.94	63	8.72	3.05	73	8.72	3.05
10.00	37	11.49	3.73	15	10.81	3.58
12.60	48	13.93	4.27	11	13.46	4.16
15.80	5	16.38	4.70			
Total	411			407		

Lower limits of size classes	No.	Mean carapace length	Mean width dimension *B*	No.	Mean carapace length	Mean width dimension *B*
0.00	5	0.85	0.77	7	0.85	0.76
1.00	11	1.08	0.96	8	1.08	0.95
1.26	8	1.15	1.31	11	1.43	1.26
1.58	11	1.75	1.54	16	1.83	1.62
2.00	17	2.25	1.95	21	2.30	1.99
2.51	16	2.86	2.42	32	2.84	2.42
3.16	10	3.51	2.91	34	3.50	2.97
3.98	4	4.44	3.71	32	4.43	3.70
5.01	2	5.81	4.65	10	5.44	4.47
6.31	30	7.41	5.91	19	7.38	5.86
7.94	33	8.84	6.94	20	9.13	7.07
10.00	36	11.49	8.72	18	11.12	8.30
12.60	46	13.96	10.34	6	13.53	9.86
15.80	5	16.38	11.83			
Total	234			234		

exactly the same for both immature and mature individuals; k is greater in immature crabs. It will also be noted that with the possible exception of the most posterior measurement (carapace width H) no sex differences are observable in the growth of the carapace. That this is not the case in the growth of the abdomen and certain of the appendages will be shown in a later paper.

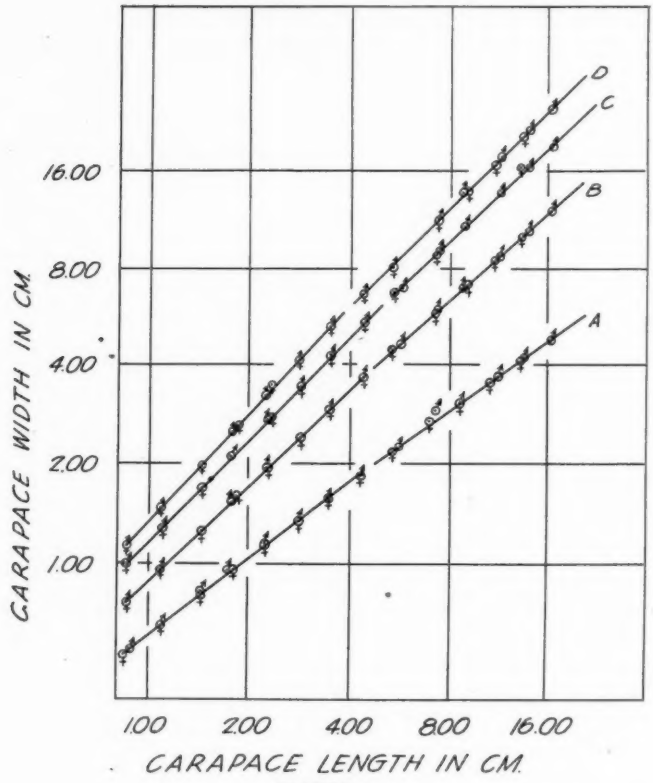

FIGURE 3

GROWTH OF THE CARAPACE OF *Cancer pagurus*, DIMENSIONS *A*, *B*, *C*, AND *D*

Figure 4 clearly shows that the growth ratio is low in the anterior and high in the posterior region of the carapace.

5. *Summary and Conclusions*

This is the first of a series of papers dealing with the relative growth of the European edible crab, *Cancer pagurus*. In this paper the growth of the carapace is studied by the method of Huxley. An analysis of eight carapace widths from anterior to posterior taken in relation to carapace length indicates an orderly range in values for the constant k from 0.70 to 1.12. This is in the form of an anterior-posterior

TABLE 2

SUMMARY OF THE RESULTS OBTAINED BY PLOTTING THE VARIOUS CARAPACE MEASUREMENTS

Dimensions	Remarks
A	The value of *k* changes from 0.76 in the smallest sizes to 0.70 in the larger sizes. No sex difference noticeable. See Table 1 and Figure 3.
B	The value of *k* changes from 0.95 in the small individuals to 0.90 in the larger ones. No sex difference noticeable. See Table 1 and Figure 3.
C	The value of *k* changes from 1.02 in small crabs to 0.94 in the larger individuals. No sex difference noticeable. See Figure 3.
D	*k* changes from 1.07 in small sizes to 1.00 (isogony) in the large sizes. No sex difference observable. See Figure 3.
E	The plot of the maximum carapace width on the maximum carapace length indicates that the value of the constant *k* changes from 1.07 to 1.00. There appears to be no sex difference in the small individuals but there is indication of a very slight possible difference in the mature crabs.
F	*k* changes from 1.12 in the small crabs to 1.03 in the large ones. There is no indication of difference in the sexes in this dimension.
G	*k* changes from 1.11 in small crabs to 1.01 in large ones. No sex difference noticeable in small sizes but there may be a slight difference in the larger sizes.
H	*k* for males has a value of 1.07 and for the females it has a value of 1.08.

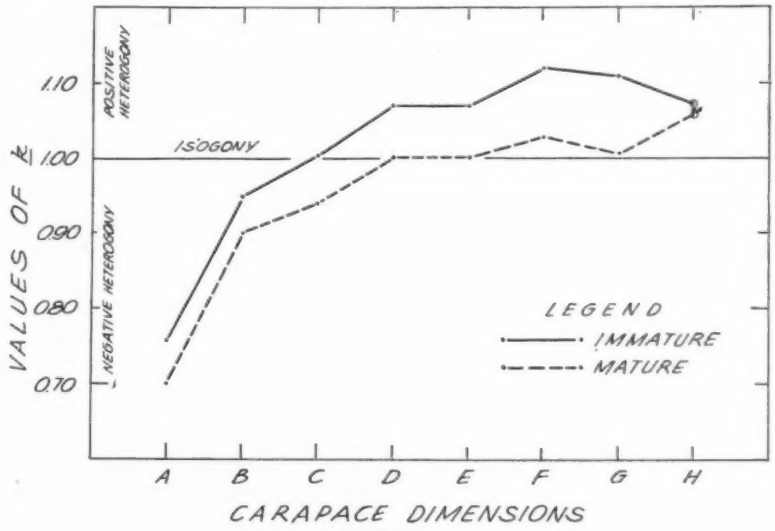

FIGURE 4

GROWTH GRADIENTS IN THE CARAPACE OF *Cancer pagarus*

gradient in which the low point is at the extreme anterior. Values for immature individuals were in general slightly higher for the same dimension than were those of mature individuals.

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THE ORGANIZATION OF PROTOPLASM: A POSSIBLE EXPERIMENTAL APPROACH[1]

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Each of the previous speakers on this program has summarized some aspect of protoplasm; the formation of structural units, the colloidal organization and behavior, and the function of protoplasmic membranes. I shall try to correlate this material in terms of organization of protoplasm as a whole, and I shall attempt to suggest experimental approaches which will enable us to begin the bridging of the gulf which separates molecular organization and interaction from protoplasmic organization into cells and tissues.

In making this attempt, I am fully aware of the trap set for those who draw too hasty analogies from the inorganic world in trying to explain how a shapeless speck of protoplasm can organize itself. Nevertheless I perhaps rush in where angels fear to tread by giving you a description of morphogenesis as it occurs in the myxomycetes and their relatives, and by speculating on the rôles of known physical and chemical phenomena in bringing the developmental changes about.

If we define organization as the arranging of the parts of a cell or the cells of a multicellular organism into definite patterns, then we have excellent material in the myxomycetes and the similar Acrasieae for studying organization. Throughout their vegetative existence these creatures belong to what may be called the initially unpolarized primary state of protoplasm in that later axiation is completely imposed by the external environment.

In contrast with such initially unpolarized states characterized by the acrasian myxameba, the myxomycete plasmodium, the myxobacterial swarm, of which more will be said later, and the *Fucus* egg, is the initially polarized state. Here asymmetrical conditions within the parent have already determined axiation, such as the side of the egg

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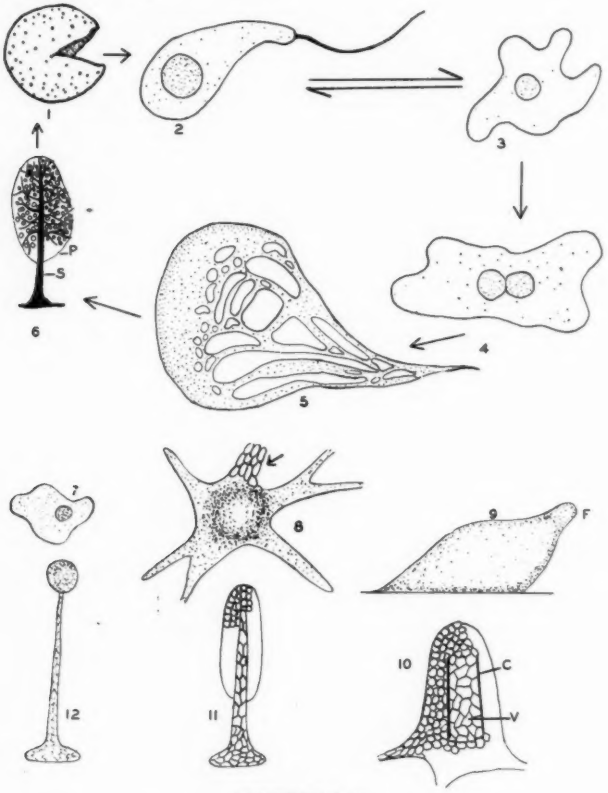
adjacent to the placenta, the one-sided position of the yolk, and an imposed shape of the egg departing from radial or spherical symmetry. Since in these cases axes of development are determined too early for convenient experimentation, we shall confine our attention to the initially unpolarized types.



FIGURES 1-12

1-6, Life cycle of a myxomycete, all figures highly diagrammatic. 1, Germinated spore. 2-3, Flagellated and ameboid forms of gamete respectively. 4, Fusion of gametes. 5, Plasmodium. 6, Fruit: *P*, peridium; *S*, stipe; a few spores are indicated among the branches of the capillitium.

7-12, Life cycle of an acrasian, *Dictyostelium discoideum*, highly diagrammatic. 7, A single myxameba. 8, Pseudoplasmodium formed by congregation of numerous myxamebae; a few are indicated by arrow. 9, Migrating pseudoplasmodium; *F*, front end, apical papilla. 10, Young fruit forming: *C*, slime sheath; *V*, vacuolated amebae of stalk. 11, Later stage; mass of undifferentiated amebae being raised on stalk. 12, Mature fruit; spore mass at top of stalk.

By tracing the life-history of a myxomycete and later of an acrasian, it can be seen why these extremely simple organisms are of such potential value for the study of protoplasmic organization.

The myxomycete spore is a convenient starting point. The spore, on being placed in water, swells, and generally within 24 hours the spore wall is either cracked open (Figure 1) or has a hole dissolved in it. Through the crack or the hole one or more small uninucleate ameboid or flagellate cells (Figure 2) slip out. An ameboid cell may suddenly put forth a flagellum at the anterior end, become elongate or comma-shaped, and swim away. A flagellate cell may just as suddenly retract the flagellum and become ameboid, and these transformations are indefinitely repeatable (Figures 2, 3). Eventually two of these gametes fuse, and with the fusion of the cytoplasms and the nuclei (Figure 4) and the retraction of the flagellum, the plasmodial stage is initiated. From now until the time of spore formation, the zygote nucleus divides a number of times, but there is no division of the cytoplasm. Therefore the original diploid cell of perhaps 10 microns diameter may continue to grow until, under suitable conditions, it covers the bottom of a large photographic tray, an aquarium, or any other sufficiently large container, and creeps over the sides and on to the table. Nevertheless the plasmodium is nothing but a huge ameba, with all the ameba's penchant for engulfing debris, its inconstancy of form, and pseudopodial creeping. While there can usually be recognized a fan-like front (Figure 5) this is by no means permanent, and any part of the plasmodium may receive into it the major part of the protoplasm and become the front.

Eventually there comes a time under conditions as yet hardly understood in which this amorphous blob of protoplasm, the plasmodium, undergoes a profound transformation. Generally, but not necessarily, it breaks up into small droplets, secretes a stalk material on which it is raised, invests itself with a stiff membrane, the peridium, and the interior protoplasm of the droplet divides into spores. Generally a mass of loose, fibrous material, the capillitium, is formed which serves to hold the spores together. With the fruiting stage achieved, the myxomycete is ready to begin the cycle anew (Figure 6).

In the Acrasieae, on the other hand, we obtain a somewhat similar life cycle, but one which has important differences. Again we may start with a spore which emits a small, uninucleate ameba (Figure 7). This ameba grows, divides repeatedly, and lives as any other small terrestrial ameba, and acrasian myxamebae have probably often been

mistaken for such. The acrasian myxameba differs from the true myxomycete myxameba in that it is never flagellate and it is never part of a sexual cycle. Now at some part of this vegetative stage, the myxamebae of an acrasian have the urge to fruit. They all start converging towards a common center or toward several common centers. The macroscopic picture is that of a small, hyaline bleb towards which are converging transparent arteries (Figure 8), the whole composed of hundreds to hundreds of thousands of formerly free-living amebae. Again unlike the myxomycete plasmodium, the cells that go to make up this pseudoplasmodium never fuse. Each cell retains its individuality but loses its independence. In *Dictyostelium discoideum* Raper, the species with the most highly organized pseudoplasmodium, the pseudoplasmodium itself moves as a whole, having a definite anterior end (Figure 9).

As the amebae converge on the central mass, a tube of polysaccharide is secreted around the interior members which become highly vacuolated, angled by mutual pressure, and secrete firm cell walls around themselves (Figure 10). More amebae at the top of this column secrete firm membranes and become vacuolated. The column increases in height by additions from the top until the entire mass of as yet undifferentiated amebae are lifted clear of the substrate by the elongation of the column (Figure 11). The completion of the process occurs when the amebae in the slimy drop at the top of the column form spores (Figure 12).

The development of the myxobacterial fructification is essentially similar, in that a slowly moving mass of bacteria embedded in a slimy matrix heaps up into fruits of a more or less complicated shape. Firm walls are secreted around masses of the bacteria which pass into a resting state.

Now here we have obviously excellent material for the study of protoplasmic organization in its most fundamental form. In the plasmodium it is impossible to pick a portion of the plasmodium and say that this will secrete stipe or peridium or capillitium, for in a few moments it will have lost its individuality in the rest of the liquid protoplasm. You can get some idea of how loosely the plasmodium is organized from an unpublished experiment of my own. I cut some large strands of vigorous *Physarum polycephalum* plasmodia. Drops of the liquid endoplasm immediately welled out, and when these drops had formed membranes around themselves, I cut the membranes and placed the secondary drops of liquid protoplasm on suitable media.

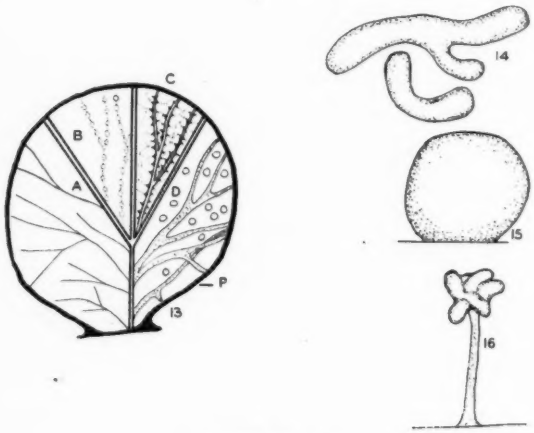
These drops reorganized themselves and grew into plasmodia indistinguishable from the parent stocks. Thus not only the plasmodium itself is a transitorily organized unit, but even the gelled walls and fluid endoplasm of the strands are completely totipotent.

Nor can we say that any particular ameba that goes to make up a *Dictyostelium* fructification has its fate predetermined as to whether it will be stalk cell or spore. Polarity occurs only after the pseudoplasmodium has been formed (Raper, 1940). Experimentally a similar condition holds for sponges and hydranths which have been dissociated into their single cells by being squeezed through a cloth bag (Wilson, 1932; Wilson and Penney, 1930; Galtsoff, 1925). The cells creep together and again differentiate into a sponge or a hydranth.

We are now back to our problem as to how protoplasm organizes itself into definite structures. I believe that our present knowledge of physical chemistry and of colloidal phenomena is sufficient to give us completely the outlines of morphogenesis in these simple forms. Admittedly, in the absence of experimental data, much of the material is speculative, but I believe it has the virtue that if the specific ascribed causes of the phenomena are thought to be wrong, there are experimental methods which will let us attack these points.

Consider the changes taking place in the fruiting of a myxomycete in terms of colloidal phenomena. If a stipe is produced, in some groups of myxomycetes it grades into the peridium, and therefore we may assume that the enzymes which form the stipe polysaccharide become concentrated at the interface of substrate and protoplasm, and to a lesser degree at the air-protoplasm interface.

If the stipe is formed interior to the mass of protoplasm, as in the case of *Stemonitis*, it is then laid down at a vacuolar interface. During the process of peridium and stipe formation a phase separation of the protoplasm occurs. Along lines (Figure 13 *A*), presumably of strain, there are formed small beaded vacuoles (Figure 13 *B*) which later enlarge, coalesce (Harper and Dodge, 1914) and at the protoplasm-vacuolar interface there is laid down material similar to that of the stipe and peridium (Figure 13 *C*). At the same time that this meshwork, the capillitium, is being deposited, progressive cleavage and phase separation of the surrounding protoplasm is also taking place. The process ends with each nucleus and its concentric mass of protoplasm separated from its neighbors, followed by protein or polysaccharide deposition around each block, dehydration of the now completed spores and capillitium (Figure 13 *D*).



FIGURES 13-16

13, Diagrammatic representation of formation of capillitium and spores in a myxomycete fruit. *P*, peridium. *A*, Formation of strain lines in the protoplasm. *B*, Beading of the lines by vacuoles. *C*, Vacuoles have coalesced to form continuous channels; protoplasm is cleaving into blocks. *D*, Completion of process. Granule-filled capillitium formed from coalesced vacuoles; a few spores shown among the capillitial strands.

14-16, Types of myxomycete fructification. 14, Plasmodiocarp. 15, Sessile sporangium. 16, Stalked fruit with convoluted sporangium as in *Physarum polycephalum*.

For the Acrasieae the same known behaviors of protoplasm are invoked, namely vacuolization of the interior cells, excretion of a polysaccharide material at the interfaces, and rounding off of the remainder of the protoplasmic material to form spores.

Assuming these things to occur through the ascribed mechanisms, what similar phenomena in the inorganic realm may be considered parallel? The concentration of material at an interface is too well-known to be discussed in detail. Many substances with which the biologist deals are surface-active, that is, they concentrate themselves at an interface. Fatty acids, proteins, lipids, all concentrate at an interface, while on the other hand sodium chloride, calcium chloride, and sugars are generally more dilute at the surface of a phase.

More difficult to conceive of is the spontaneous separation of protoplasm into two distinct phases, i.e., concentrated protoplasm, and vacuoles. Yet there are splendid models of this very behavior in colloidal chemistry. If one mixes aqueous solutions of gum arabic and gelatin in the presence of an electrolyte, there is a separation into two liquid phases, both aqueous. The heavier phase contains

most of the colloids, and if this is shaken with water, the heavier phase is seen to form small globules containing droplets of the lighter phase. These droplets of a coacervate are quite analogous to protoplasmic vacuoles. Not only dilution, but addition of salts, changes in temperature, changes in relative concentration, and a number of other factors can cause vacuolization in a coacervate droplet. The addition of lipoids to such coacervates causes the formation of vacuolated droplets with membranes remarkably similar in their behavior to the membranes of cells. (Bungenberg De Jong, 1936; Bungenberg De Jong and Bonner, 1935).

It would be an easy step from the behavior of such colloidal mixtures, simple as they are, to the more delicately invoked reactions of the tremendously complex colloidal mixture known as protoplasm. The oxygen, water, or CO_2 gradient from the periphery to the center of a heap of myxamebae may well cause the phase separation and vacuolization of the interior ones (Figure 10); by the same token the dehydration of the protoplasm and the pressure of the drying peridium may so change the concentration of water or other material in the myxomycete fructification that a phase separation of vacuole and concentrated protoplasm occurs (Figure 13) as indeed we know it does in the simple coacervate systems I have described.

The objection may be brought forth at this point that after all only the heterogeneity of a completed structure has been taken into account; how would one account for the differences in shape of the entire fruits of myxomycete fructifications of different species?

Some myxomycetes fruit as plasmodiocarps (Figure 14), that is the protoplasm of the plasmodium merely becomes invested with a peridium with only slight changes of shape. Others break up into small sessile spheres (Figure 15), and still others form fruits on more or less elongate stiped (Figure 16).

I believe that one need not invoke special form-determining factors of a mysterious nature to account for these diverse shapes, but merely the species-specific chemical nature of the materials secreted at the surface of the protoplasm. If the material has a low surface tension, we should expect the plasmodium to fruit as a plasmodiocarp, the surface tension being on the whole too low to overcome the cohesiveness of the protoplasm and the adhesion to the substrate. A higher surface tension would produce sessile spheres, and finally if the material is secreted preferentially at the substrate-protoplasm interface, we should expect a stiped form.

If this is true, then it should be possible to make a normally stiped form sessile or even plasmodiocarpous by decreasing the sharpness of the transition from the protoplasmic phase to the outer environment, in other words, by lowering the surface tension. Observations of my own show that this does occur when the plasmodium fruits under conditions of excessive moisture (unpublished results). If the plasmodium has spread from the moist substrate upward along the side of the dry vessel wall, there is a beautiful transition from the plasmodiocarpous type of fruit at the bottom under conditions of extreme moisture, through sessile forms, to stalked forms at the top. I have observed this transition in such normally stalked forms as *Didymium squamulosum, Physarum polycephalum,* and the usually stalked *Badhamia foliicola.* In some cases, by the manner in which myxomycetes are differentiated, this would be equivalent to a change in species!

I stated previously that metabolic gradients from the exterior to the interior of the protoplasmic mass, the pressure of the drying peridium, and the consequent squeezing out of water could be responsible for vacuolization, hence capillitial formation, since the capillitium-material is laid down at the vacuolar-protoplasm interface. If this is true, then as a corollary, we should expect a somewhat reduced capillitium in fruits formed under conditions of excessive moisture, and an overdeveloped one in fruits formed under conditions of extreme dryness. The few observations I have made, though far from complete, give the impression that myxomycetes fruiting under extremely moist conditions have a weakly developed capillitium compared with the normal. Conversely, on searching the literature, I found that Brandza in 1926 (1926, *a*, *b*) had observed a much greater development of the capillitial threads in myxomycetes fruiting in exposed, dry situations, as compared with the fruiting of these same myxomycetes under more nearly normal conditions.

It is interesting to note that Henrici (1928) found the expected effects of surface tension depressors and augmentors on the shape of the *B. coli* cell. This usually short, plump rod if grown in ricinoleate solution, a surface tension depressant, formed elongated cells. If the organism were grown in broth containing calcium chloride, which raises the surface tension, the cells were almost spherical in shape.

At this point I should like to bring to your attention the possible rôle of surfaces in orienting protoplasm, and consequently in the development of the cell or tissue. This is a rôle which I believe has been largely overlooked by biologists.

It is true that we can explain many surface effects as blocking of gradients. As an example, Whitaker (1940*a*) has shown that under certain conditions the *Fucus* egg forms its rhizoid adjacent to the surface on which it rests because of accumulation near the surface of acid metabolites from the egg, whereas in the surrounding liquid they can diffuse away.

But there are data existing which indicate that a substrate surface can exert a much more direct influence on the orientation of the protoplasm and thereby the polarity of the cell. Thus Taylor and Strickland (1935) have found that the viability of Colpoda cysts is to some degree dependent on the type of surface on which the organisms encyst. Cysts formed on cellophane are more viable than those on mica with glass and quartz intermediate. Zobell and Anderson (1936) have also found that the growth of bacteria in sea water is proportional to the available surface to which they may adhere. It has been known for a long time that the cells of many tissue culture transplants, if not given a substrate to which they can adhere, round up and die without growth, and the same is true of myxomycete plasmodia and many amebae.

While some of these phenomena may be caused by micronutrients leaching out of the substrate, or by oligodynamic effects, it would be difficult to ascribe all of them to diffusible substances. We must realize that a surface on the micro-scale is not a mathematical plane, but a layer of more or less oriented molecules or atoms with fields of force which in some instances may be quite powerful, and may extend to almost microscopic distances from the bulk phase. As one example out of many from the field of colloids, Ornstein and Kast (1933) found that the surface of a glass slide exerted an orienting influence on cholesterol ester molecules for a distance of 0.2 microns. Between a cover glass and a slide there would be a total orienting influence extending 0.4 microns, practically a microscopic distance. It may not be too far afield to expect that the beneficial effects of contact may be due to the forces of the substrate molecules acting across the submicroscopic cell membrane and orienting particles into a more efficient arrangement for carrying on metabolism, particularly in cells whose colloidal structure is such that the particles are only weakly structurally organized with respect to each other.

If this is the case, then we might expect that an orientation of the medium would produce a reflected orientation in cells in that medium. Such orientation phenomena have been demonstrated by Weiss in tissue

cultures. If chick mesenchyme is grown on a plasma clot prepared in the usual manner, the cells migrate out randomly from the inoculum, and show the usual irregular, inconstant shape of mesenchyme cells. But if the clot has been stretched or stroked during gelation, producing an orientation of its micelles, the mesenchyme cells move along these lines of orientation, and what is most exciting, differentiate into the connective tissue cells typical of tendon. A similar behavior has been demonstrated for nerve fibers in vitro (Weiss, 1933, 1934, 1939).

Recently Mr. Roger Stanier at the Hopkins Marine Station has demonstrated that myxobacteria, which incidentally cannot be grown in liquid culture, when fruiting on an agar which has been placed under tension or compression, form their fruits with respect to the lines of stress. Forms which on an unoriented substrate fruit as small spherical bodies, fruit on an oriented substrate as sausage-shaped masses.

It may seem to you that so far I have stressed the surface phenomena as playing the chief rôle in protoplasmic organization as compared with gradients. I should not care to leave you with this impression, for I felt that it was not necessary to stress gradient effects which are already so well known to you through the work of Child and his school. In the initiation of the pseudoplasmodium of the Acrasieae, it must be a gradient, i.e., a direct chemical influence, which starts the movement of amebae toward a common center (Figure 8). Whitaker has shown that gradients of acidity can polarize the *Fucus* egg with respect to formation of the rhizoid as well as the physical gradients of temperature, and light, and that imposed by centrifugation (Whitaker, 1935, 1938; Whitaker and Lowrance, 1936) (summarized by Whitaker, 1940*b*).

We must not forget however that there is an intimate connection between the physical phase boundaries and chemical gradients. From a boundary a gradient of concentration of materials may extend into the cell, and conversely, along a gradient there may be one or more places of optimal or critical concentration for phase separation as in the formation of coacervates, and the vacuolization of the interior amebae of a young acrasian fruit.

I think the mistake has too often been made in the use of the gradient concept in considering the secondary effects of any particular chemical gradient as increasing regularly from one pole of the organism to the other along with the primary gradient. Both biochemistry and colloid chemistry show this to be untrue in many cases. Thus the action of most enzymes along a gradient of hydrogen ion concentration increases

with increasing pH, reaches an optimum, and then falls off. The same is true of the behavior of proteins subjected to a hydrogen ion gradient. There are one or more points, i.e., isoelectric points, where the properties of swelling, solubility, and sensitivity to precipitation are at a maximum or a minimum. Along an electrolyte gradient there are one or more points which are optimal for coacervation, dispersion, and swelling of colloids. The same holds true for many physiologically active substances. If the appropriate plant tissues are subjected to an auxin gradient, there is an increase of growth corresponding to increasing auxin concentration, but eventually a point is reached where increasing the auxin concentration no longer increases the response, but actually suppresses it. It is interesting to note that the optimal auxin concentration varies enormously for the different plant organs (Thimann, 1938). Therefore in protoplasm organized with respect to a gradient we may expect more or less sharp qualitative changes in behavior at optimal points, as well as a graded over-all quantitative response.

The conclusion to which I am leading is the application of what may be called the principle of self-increasing complexity. That is the idea that once a certain degree of complexity is introduced into a medium, the complexity will increase under its own momentum, physically, chemically, or both. As a simple example we may take an ordinary concentrated soap solution prepared from the two components, sodium stearate and water. Immediately on mixing these there are formed several more components, the ions of the salt and of water as well as the free sodium hydroxide and stearic acid. If the mixture is carried along a temperature gradient, there may be one or more places where two or more phases are formed, one or more of which may be truly liquid, the others liquid crystalline or crystalline, these last two having a spatial orientation of the components. The system has increased enormously in complexity, and the addition of another substance could increase the complexity to another magnitude.

For a biological example analysed somewhat crudely in the same way, we may consider the *Fucus* egg. Pressure applied asymmetrically, imposed chemical or physical gradients, could produce a preferred orientation of some important substance within the egg. Thus one end becomes rhizoid primordium, the other, the frond. The existence of a gradient from rhizoid to frond end would produce secondary gradients of metabolic activity. As a result of all these factors a critical state is reached where there is a change in the aggregation of

the protein micelles of the protoplasm, the nucleus disintegrates, and a spindle, which may be an oriented protein structure known as a tactoid, is formed. The chromosomes move apart, and at the equator of the tactoid which may be a special type of coacervate (Langmuir, 1938), the critical conditions for the deposition of polysaccharide are achieved. A cross-wall is thereby laid down and the egg is two-celled.

Besides the original induced gradient, we have to consider at least two more gradients, those from the new cross-wall into the cells. There are probably many more, since the new cell membranes and cross-walls would cause a selective hindrance of the diffusion of materials. The same process being repeated gives a structure which is already becoming highly complex, and I need not carry the story further to illustrate the principle of self-increasing complexity.

Therefore I propose the following as the simplest working hypothesis of organization: given just the number of asymmetrical conditions in the environment, whether ovary or ocean, to determine the main axes, the chemical complexity of protoplasm is sufficient to evoke further spatial patterning. This in turn increases the complexity of the gradients and phases and so evokes a still more complex patterning, the whole cycle repeating itself until the system has reached a condition of dynamic stability or of rigidity as in fructification. If we take into account the chemical complexity of protoplasm as compared with inorganic systems, we do not have to postulate form-determining factors any more vague than the proteins, carbohydrates, and other well-known substances which are chemically unique for each group of organisms and to a certain degree for each species.

Finally, if we wish to make use of experimental approaches such as induced orientation, imposed surface tension, induced gradients, and others, it must not be forgotten that we are experimenting with the most complex chemical and physical medium on earth. It is necessary to distinguish between the direct expected effect of an experimental factor and the final effect as the result of a long chain of obscure reactions which may mask completely the effect for which we are looking.

Since the presentation of this paper the *Third Growth Symposium* has been published (*Growth,* 5, *Supplement,* July, 1941). Two included papers of particular interest in connection with protoplasmic organization are: K. B. Raper's *Developmental patterns in simple slime moulds* (41-76), and F. O. Schmitt's *Some protein patterns in cells* (1-20).

On the basis of Langmuir's concept of a tactoid as an oriented coacervate, it would be expected that polysaccharides promoting the formation of protein-containing coacervates should also promote the formation of tactoids in solutions of linear proteins. A few hitherto unpublished qualitative experiments of the writer's indicate that this may well be the case.

The protein used was a solution of tobacco mosaic at pH 7 (kindly furnished by Dr. I. Fankuchen; cf. Bernal and Fankuchen, *J. Gen. Physiol.*, **25**, 111-165, 1941). This solution, when shaken, separated into two layers, the process taking several days. With some difficulty tactoid formation could be demonstrated under the polarizing microscope. On very slight dilution tactoids either were not formed, or if present, disappeared. However the addition of small quantities of approximately one per cent solutions of any one of a number of common polysaccharides (gum arabic, starch, glycogen, etc.) caused the immediate formation of extremely long, very delicate and flexible birefringent fibers. These fibers could be considered as dehydrated elongated tactoids. The most interesting results were obtained with a polysaccharide isolated from the plasmodium of the myxomycete, *Physarum polycephalum*.

After the mixing of a drop of dilute myxomycete polysaccharide solution with a drop of tobacco mosaic there was an immediate separation into two phases, one highly birefringent, the other weakly so. Within a few minutes the rounded boundaries of the strongly birefringent phase became cusp-shaped, and within a half-hour to an hour the birefringent drops had broken up into spindle-shaped bodies of various sizes and degrees of elongation. Some were of comparatively tremendous size, perhaps 200 microns or longer. Twinning and fusion of these structures were frequently observed. Unlike the original tobacco mosaic sample, the mixture formed tactoids very readily.

What bearing protein-carbohydrate complexes may have on spindle formation within the cell remains to be seen, and further experiments, at least by the writer, will probably have to await the return of more nearly normal conditions.

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THE TEACHING OF ONCOLOGY

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The present thesis is designed to: (*a*) examine the question of the desirability of a special department in medical schools for the teaching of oncology; (*b*) give an outline of the organization of such a course and its relation to the teaching of other subjects; (*c*) indicate how a special department of oncology can be organized for this teaching and how it can be integrated into the staff of the hospital connected with a medical school; and (*d*) give experiences with such a department for the past three and one-half years in a particular medical school, namely, the Hahnemann Medical College and Hospital of Philadelphia.

A. DESIRABILITY OF AN INTEGRATED COURSE IN ONCOLOGY

1. *The Tumor Clinic in Hospitals*

For a number of years individuals interested in oncology have urged the formal organization of tumor clinics in the various general hospitals of the country. For ten or more years this idea has been fostered and encouraged by the American Medical Association, the American College of Surgeons, the American Society for the Control of Cancer, many State and local cancer committees, and other interested organizations. The reasons for the establishment of tumor clinics are as follows:

(*a*) Tumors occur in any and all regions of the body and therefore require the use of highly technically developed instruments and apparatus for their early detection.

(*b*) The possibility of complete removal and/or destruction of malignant tumors is in direct ratio to the early time at which they give symptoms and which brings patients for examination.

(*c*) The treatment of patients with malignancies in various parts of the body requires different techniques depending on the numerous variables presented by each individual.

(*d*) The basic specialists recommended for the conduct of a tumor

clinic are pathologist, surgeon, roentgenologist. The pathologist is needed for his experience in the gross and microscopic diagnosis of tumors, his judgment as to type of tumor and general growth propensities. In addition he contributes his knowledge of the course and end results of tumors from his general training and autopsy experience. The surgeon is needed for his diagnostic skill, his technique in removal and knowledge of possibilities. The roentgenologist is needed for diagnosis by roentgenograms and other means and for treatment. The latter includes judgment as to the sole use of irradiation or in combination with surgery. To this basic staff are added an internist, for the proper care of a cancer patient includes general supervision and treatment and the various specialists in dermatology, urology, bronchoscopy, otolaryngology, stomatology, neurology and the rest. Thus there are brought to bear upon each individual patient the combined skills and experiences of numerous specialists. In addition, tumor clinics teach interns, residents, and the chiefs themselves. The use of the tumor clinic in the teaching of medical students will be briefly discussed later.

If it is well recognized that correlation and integration are necessary in the handling of tumor patients in general hospitals, as it is and has been for many years, it is no great extension to carry this thought to the teaching of oncology to undergraduates.

2. *Some Reasons for a Course in Oncology*

Whatever the reasons and they will not be discussed here, the incidence of tumors is increasing steadily. Cancer has ascended to the position of second cause of death with approximately 150,000 dying of the disease each year during the past few years. Estimates vary as to the actual number of cases per death from two to three, but the number is sufficient to warrant the most concentrated attention. Unlike that of most other diseases the mortality of untreated cancer is 100 per cent (1).

As at present carried out in most medical schools, the teaching of oncology is done in various departments usually beginning in pathology. But in the usual course in pathology many of the organs which are particularly important in oncology are not heavily stressed such as the breast, the prostate, the thyroid, the salivary glands, the rectum, etc. Ordinarily the time allotted to pathology is expended mostly on the heart, the liver, the kidneys and other organs. This may be necessary for time reasons but it is inadequate for oncology (2). These

omissions must be filled in at some time and can be done adequately in a special course in oncology.

From pathology onward students are further instructed in the otolaryngological, ophthalmological, dermatological, internal medical, genito-urinary, surgical and radiological departments, indeed, in any and all of the specialties. This teaching leaves loose ends and is necessarily somewhat disjointed. Experience has shown that most students in their third and fourth years have not as yet reached a degree of proficiency in medicine, nor in science in general so that they can by their own efforts tie these loose ends together into one coördinated whole. Oncology, in this respect, is an ideal subject from a pedagogical point of view, for just this type of teaching. Even though many make the statement that cancer is not a single disease, but a number of different diseases, it can be stated with equal truth that there are a few common denominators in all cancers and that all of the different kinds can be recorded under a few headings. This, as is clear, depends upon the point of view, whether clinical, pathological, biological and/or experimental, all of which should be presented as related stories.

Even in its clinical aspects there is a supreme unifying concept. The goal of oncological practice is early diagnosis and prompt and radical treatment. It can be said that the time at which patients should consult their physicians for tumors is in the stages in which the diagnosis cannot be made by ordinary means. Put into other words, the differential diagnosis is of the utmost importance in separating tumors in their early stages from other conditions. To become expert in oncology, therefore, predicates first becoming a good general doctor. No more need be said about the necessity of correlating all the stray bits of knowledge which the students have acquired, of filling the gaps and adding to their information. They must be taught to develop "suspicious" minds, and for oncology, a knowledge of all the special means at hand for diagnosis, so that they will instantly leap into the student's consciousness as he conducts his history taking and physical examination. To stress another point, as an argument for the desirability for special courses in oncology the experience of the Division of Cancer Control of the Department of Health of the Commonwealth of Pennsylvania during its brief two years' existence can be cited (3). In about 20,000 cases the grand average delay time between discovery of symptoms and radical treatment was about 15 months. Four of these months were due to delay on the part of physi-

cians. Extenuating circumstances existed in some, such as diabetes, hypertension, nephritis, etc., but nevertheless there is a woeful lack of that "suspicious" mind referred to above. Obviously the place to begin implanting it is in the medical school.

Among still other reasons, is one which is becoming increasingly important. The last five or more years has seen an increasing amount of education in medical matters offered to the general public. In some instances this has been requested by the public; in other instances pressure has been applied to make the public "health conscious," "tuberculosis conscious," "diabetes conscious," or "cancer conscious" or whatever happens to be the enthusiasm of the group endeavoring to do the educating. Newspapers have opened their pages to much more of medical matters than formerly (4). This is equally true for the radio. Working in both directions as many such processes do, more and more people become interested and more and more demand is made for such programs. It is all reflected in more pointed questions asked in the doctors' consulting rooms and in a greater and greater demand for speakers for lay audiences. Needless to say, to do these things well in cancer, as they must be done, requires a degree of correlation somewhat higher than can ordinarily be acquired without specialized courses in the subject (5).

B. Outline of a Course in Oncology

Just as the roots of cancer ramify in the unfortunate victims in all directions, so do the roots of knowledge of the disease ramify into many places. The mother-subject is biology, and the disciplines of biology related most intimately to cancer are genetics and experimental embryology, for they deal directly with the problems of growth and development (6). Since cancer is primarily a growth process, the only possible comparison is with normal growth and development. But biology is having chemistry and physics introduced into it very rapidly. Mathematics in the form of statistics have been there a long time (7). So the chemistry and physics of growth and development must be reviewed insofar as facts permit and theories allow. But there is scarcely any subject which cannot contribute its share to cancer knowledge. Psychology is of extreme importance in the treatment of cancer patients. Neurology, agriculture, forestry, fisheries, botany, etc., all have contributed their share.

The subject called semantics (8) is of high importance and we may state emphatically that it has hardly been introduced, for a

cursory survey of nomenclature and words used in oncology shows a woeful lack of attention to this important branch of knowledge. The nomenclature of tumors is weird and confusing. In many instances it seems to follow the recommendation made by someone that "where you cannot clarify, mystify"; or, "give it a name and it is explained"; or even, "it is a so and so because I say so." While awaiting reform in this portion of oncology it is highly essential that students be led through the maze of contradictions of language and of classification so that a clear conception is obtained of processes rather than statics. Sometimes the histological approach is best, at other times other disciplines may be used for the clarification.

During the last few years an old subject called "logic" has received a new impetus in the form of what is called "symbolic logic". An ardent and creative champion of this method of approach is J. H. Woodger (9) who, in numerous publications, has shown its utility. He has even introduced qualitative terms into equations!! The attempt has led, of course, to the raised eyebrows of mathematicians, engineers, and others who are accustomed to using quantitative terms in their mathematical equations. But Woodger finds that none of our present systems of mathematics is adequate for biology, and therefore has attempted, at least, to devise a new system especially adapted to the needs of the subject. The philosophic implications are far-reaching indeed, not only for biology, but for all science. Until further developments appear along these lines of thought as applied to oncology, we may regard the attempts not only as interesting and promising, but as having yielded several important applications. As in all other mathematics, nothing more is gotten out than is put in; that is to say, when the facts obtained by observation and/or experiment are analyzed mathematically, no new facts emerge, but the facts put in are ofttimes rearranged into much more intelligible and useful form. New implications and new deductions appear, many of which would not have been manifest had the mathematical treatment not been applied. Furthermore, in any given subject there are gaps in knowledge and oncology puts particular emphasis on the gaps. The treatment of facts, observational or experimental, by Woodger's methods has clearly shown in several instances exactly where the gaps in knowledge are and thus has provided definite questions for immediate experimental attack.

As a further introduction, certain philosophical considerations are mentioned. While this may seem far afield from technical medicine,

not to say oncology, nevertheless most individuals work and think according to some philosophy whether they admit it or not, and many individuals who do not have definite ideas of this kind find confusion in the correlation which is so necessary in medicine. Direct questioning of students has elicited two statements: (*a*) less than one-fourth of each class has been taught any of the philosophical backgrounds and most of those who have been exposed to such teaching have either forgotten it, have ignored it, or are not able to apply it; and (*b*) at least one-half of the students regard an hour or two devoted to the philosophy of science as extremely useful and as having opened fields of thought and methods of correlating their various studies which have not occurred to them before. This, in my opinion, justifies the time taken for these remarks if no other good comes of it. It may prevent at least a few of the students who become specialists from looking at a patient as existing merely above the clavicles, if they are otolaryngologists; or as a uterus, two tubes and two ovaries on stilts if they become gynecologists. If an organism is a system devoid of isolated events, then indeed it is necessary for good doctors to look upon their patients as organisms, recognizing that a change in one part of the body inevitably leads to changes and adjustments in other parts which must be considered in the management of the patient as a whole. The adjustments of the patient's whole so necessary following radical operation and/or irradiation for cancer are extremely important considerations.

As a beginning to the remarks on the philosophy of science, the students are reminded that it is possible to predict with practical accuracy that the offspring of a given species will turn into a replica of its parents under the right environmental conditions; in fact, at any particular stage of development of a thousand or more embryos of a given species, under the same environmental conditions, there will be more or less close uniformity between each of the embryos, except for occasional mutations. Even when the major growth period of any animal is completed, i.e., when it reaches adult size (in those that reach a definite size) the expressions of growth and developmental activities do not cease, for there is always the necessity for repair, replacement, physiological adaptation, and at times, regeneration.

The concept of *ORDER* in development is introduced. It is pointed out that it is hardly an accident that many of the great biologists of all times have been philosophers. They have noted this *order* and have pondered deeply on its meaning and significance.

The Aristotelian four principles for the understanding of Nature are briefly rehearsed and the statement made that of the "efficient," the "material," the "final," and the "formal" causes, modern science can recognize only two, viz., the efficient and the material, or in general terminology, the physics and chemistry (10).

Harvey's views are expressed. He states, it will be remembered, that the "efficient cause of the chicken is hard to be found out." It certainly is, but experiments on growth and development are headed for this goal (11).

Hormism or "psychological biology" is mentioned: "a physiological tale needs also a psychological one with it" (12). It is emphasized that the coining of words with psychological backgrounds does not explain phenomena in a way which makes them controllable. Explanations in these terms may be convenient temporarily and may even seem simple, but in the long run they must be replaced by words of general import in science, that is, in physico-chemical terms. This may be more difficult at any given time, but nevertheless finally it must be done. Concepts continually change as more information is gathered. A theory is developed as a tool for immediate use to be replaced by sharper and better ones as they are invented. No business firm can afford to make its gadgets with obsolete equipment.

Teleology is discussed briefly, and the effect before the cause. It is emphasized that it can hardly be of use to practicing physicians to think that a predetermined end is mysteriously operating to pull forward and guide the paths through which growth and development take place. The purpose, the teleology, of a fertilized ovum's developing into a completed new individual is to develop into a completed new individual. Or, it is planned that the fertilized ovum develop into a new individual and all individual processes in this development are subservient to this end. This is not science! As well say that the purpose of hydrogen and oxygen is to produce water; that a present effect is produced by a future cause. Lawrence J. Henderson's book, *The Fitness of the Environment,* is recommended as a most helpful antidote to this kind of unscientific thought (13). Naturally, Immanuel Kant is mentioned and how he helped in separating metaphysics from science and his insistence on the part played by the person doing the thinking (14). The famous controversy between Jacques Loeb and H. S. Jennings is of great interest. Loeb's tropisms need no psychological factors; Jennings thinks psychological factors are legiti-

mate if made subject to scientific investigation as to their origins and mechanisms (15).

Organicism or "we murder to dissect" is also mentioned, only to be cast aside as an insufficient philosophy for physicians.

There are then introduced the ideas in *Science and the Modern World* and other writings by A. N. Whitehead (16). The concept of the Organism as a Whole is emphasized. It is taught that an organism is a system devoid of isolated events (17), that no organism is the sum of its parts but that the parts fitting together produce a new and different entity. In fact all things can be regarded as organisms. The difference between the living and the dead may be looked upon as differences in degrees of organization and dynamics. While the complexity of living matter makes it difficult to think of it in physico-chemical terms, nevertheless in laboratory work, it is the only permissible way (18). Quoting Hammett (19), "Science is the accumulation and application of data on the relations between things; between states; and between events; and on relations between things, states, and events." This objective definition can be immediately changed to a subjective one really suitable for physicians by adding "for the benefit of mankind".

It is explicitly stated, however, that the above does not exclude other theories. As doctors of medicine, the students are called upon to advise and treat individual patients (and their relatives), and this introduces an additional philosophical necessity. "The courts of heaven do not resound with mathematical phrases, or organizers of the first degree, or milligrams per-cent" (Wm. James); or, as Needham put it (18), science gives "a theory of the world and not a pocket edition of the world itself." But the "art" of medicine as it is so loosely called, must not contradict nor nullify that part which is the legitimate science of medicine (20).

Finally, the principle of indeterminancy is mentioned (Schrödinger, Heisenberg) as an additional prod to the students to use statistical methods in their work and to remember there is a "probable error" not only in their thinking but also in their data. If the new physics is limiting, as it is, the possibilities of our knowledge to approximations and relativities and, what is more to the point, to comparisons of velocities and rates and acceleration as so clearly stated by Eddington (21), are the behaviors of atoms and molecules, indeed of electrons, as statistical approximations, any different fundamentally from the behavior of living organisms in which the variations discovered in

experiment are greater because the number of individuals measured is so much smaller? As instruments become more delicate and methods finer, less and less numbers of electrons, atoms, molecules, organisms, can be measured, and natural laws become statistical approximations (Reichenbach, 22). J. Arthur Thompson in his *The System of Animate Nature* (23) stresses the individuality of organisms. In view of the teachings of physics shall we not include the molecule, atom and electron into one glorious Whole? Each particle of Nature is an individual and as particles become associated with others, the association in its turn becomes a Whole with all that this implies.

Science deals with the measurement of these Wholes and the parts. It must have both. Organicism is inadequate.

Return to more technical matters is made by stating that study of growth and development may be begun at many points, but for convenience the fertilized ovum may be chosen. Not that growth and development do not take place before this, for both the ovum and spermatozoon are highly differentiated structures. Their development may be regarded as multiplication and differentiation wherein the highly important reduction of chromosomes takes place, and finally separation of the mature cells. In contrast to politicians, legislators, some sociologists and others who regard human beings as just men and women, physicians should consider them under four headings, viz., men, women, spermatozoa, and ova, the latter two added obviously because the physician is certainly interested in the inheritance of his patients and in turn in their ability to produce sound spermatozoa and ova (Pummett).

Parthenogenesis is discussed (24). It is of interest in the theory of development of certain tumors such as teratomas, dermoids and the like. In point of number, more living organisms reproduce asexually. Sex introduces a complication but is useful in mixing stocks and helping render them more fit to survive. In all organisms tested parthenogenesis of ova has been found possible (24). Fatherless rabbits are well known (25). The human ovum has divided at least once parthenogenetically in the laboratory (26). Spermatozoa of kinds which lend themselves to experiment have also developed parthenogenetically (27). Therefore certain ovarian and testicular tumors may have their origin in parthenogenetically developed ova or spermatozoa. Others may come, of course, from the unfortunate ones of twins, triplets, quadruplets, or perhaps even quintuplets.

The word "growth" is too inclusive for good practice. There are

growth in size, growth in number, growth in specialization, growth in complexity, differential growth, etc., even, perhaps growth in intelligence. The kind of "growth" under consideration in any particular instance should be specified.

The "organismal" theory emphasizes that the most spectacular characteristic of normal growth and development is that wherein all of the complicated processes head toward the completion of a whole, thoroughly integrated organism. The cell theory in its classical form states that multicellular organisms develop by the multiplication of cells and evolve by the addition of cells. The organismal theory believes that the protoplasm of organisms is continuous, that it differentiates into various kinds and that its subdivision into cells is only one of the numerous differentiations which it undergoes. Examples of large, competent organisms without cell walls but with many nuclei are given, e.g., the siphonaceous algae. Therefore it is stated that the cell theory is not a generalization but a special case. It is better to speak of the activity of protoplasm than the activity of cells. deBarry's way of putting it is emphasized, namely, "the plant (animal) builds cells and not cells the plant (animal)." Certain tumors are composed of nucleated masses of protoplasm, i.e., syncytiae (28).

As stated, growth processes both normal and abnormal may be analyzed in many different ways. For various reasons it is begun by speaking of cell multiplication, cell differentiation, and cell organization. Since there are endless differences in the way in which these terms are defined and used, very careful definition of them is given. Multiplication of cells means their increase in number. Differentiation of cells means the transformation from a more general to a more special type of cell. Anatomically, the latter is a reflection of chemical rearrangement within cells during which they develop a chemical composition characteristic of a particular, special kind such as muscle, skin, bone, etc.

Organization is the building up and maintenance of parts or organs by the harmonious growing together of a number of variously differentiated cells into a new individual in a specific frame characteristic of the lineage.

Multiplication is the least specific, differentiation more, and organization the most specific, i.e., specialized, of the three processes. Both differentiation and organization are performed not as processes completing isolated parts and joining them as a sum, but as processes which build parts into the frame of a whole. Probably multiplica-

tion, differentiation, organization were evolved in that order as the means by which living organisms in general evolved from the simple to the complex. Certainly they are the means by which a single individual evolves in his ontogeny from the simple to the complex, from the general to the specific. It is said that those properties acquired latest in evolution are the most sensitive to disturbance. If so, then malignancy is an example, for organization (in the above sense of the word) is completely gone and differentiation disturbed.

Multiplication, differentiation, and organization serve different functions. They are independent, yet dependent processes. They can be thrown out of gear or caused each to proceed in the absence of the others by suitable changes in the surroundings, such as by cold, heat, interposition of one process on another, e.g., repair or regeneration, various chemical groups, e.g., sulfhydryl and others. But that there are interrelations is obvious enough. Cells cannot differentiate unless they are there and they appear by multiplication. They cannot organize unless and until they have differentiated. The relationship may be expressed this way:

"Cells multiply"
"Cells differentiate after they have multiplied"
"Cells organize after they have multiplied and differentiated"

In this table it is very easy to plunge off the deep end by substituting a few words. Students are invited to try this and allow their thoughts to swim around seeking a safe landing place.

"Cells multiply"
"Cells differentiate because they have multiplied"
"Cells organize because they have multiplied and differentiated"

or in this wise:

"Cells multiply in order to differentiate"
"Cells differentiate in order to organize"

or:

"Cells differentiate and organize because they have multiplied"

The students are then invited to carry these thoughts to the further statement which opens a wide vista and suggests endless experiments, viz.,

"Cells multiply because they do not differentiate and organize"

They recognize this immediately as the long sought "Cause of

Cancer." Cancer cells continue to multiply because they do not differentiate properly and do not organize at all. Their attention is immediately centered on the organization problem which is today the key-problem of all biology. It is an exciting episode in the classroom routine. The students also recognize it as an example of practical semantics: the "meaning" and implications of statements.

1. *Proliferation* (29)

Mitosis is a basic process by which protoplasmic masses are increased in number. The fundamental scheme by which the chromosomes are divided is the same for all living things which divide by mitosis. It is a reasonable assumption that whenever such basic processes are similar in many different species of animals and plants, common chemical mechanisms underlie the processes.

This remark gives opportunity for rehearsing in detail the experiments in which it was discovered that the sulfhydryl group is active in proliferation (30). It is done for several reasons: (*a*) the experiments illustrate the applicability of investigations in plants to human problems for the original experiments were done with peas, beans, corn, and onions; (*b*) they illustrate the resourcefulness which must be used in such experiments; (*c*) they illustrate the necessity of picking out phenomena common to all living things when plans of experiments of this kind are being made; (*d*) they show how the application of this knowledge has been of use in the practice of medicine; (*e*) they illustrate that a good way of asking the question in research is to put it in such wise that the answer when obtained, whether negative or positive, will be of value.

Further points about mitosis are emphasized such as the distinct antagonism which is marked even though not absolute in tissues, between differentiation and proliferation. Cells do not divide at the peak of their special physiological function, and conversely, dividing cells do not exhibit physiological functions except the general ones necessary for life itself, e.g., respiration (31). Mitotic capacity is retained only by a certain proportion of cells in any particular group, the younger the developmental stage of the tissue, the larger the proportion.

These latter facts are brought to bear upon the problem of the histological grading of tumors. It is stated that the less differentiated the cells of a tumor are, as evidenced anatomically, the more rapidly in general does the tumor mass grow, because the mass contains more

cells capable of division. The converse also is true. Practically speaking, histological tumor grading is of little or no value to an individual patient, can be used only in statistical studies and must never be used as a basis for reduction of radicalness in treatment. The only difference between a malignancy which grows more slowly and one which grows faster is a short interval of time. Treatment demands that the complete mass be removed or destroyed for cure, irrespective of name, site, histological appearance, or any other such criteria so long as malignancy is established (32).

When mitosis is about to occur, preparatory anatomical changes can be seen which are the mirror of chemical and physical rearrangements. These become more pronounced and definite as mitosis proceeds and culminate with the division of the mother cell into two daughter cells. It is emphasized that while the physical and chemical characteristics of the daughter cells immediately after division differ from the mother cell before it divided, nevertheless there is a chemical continuity which insures the lineage and the type of cell. The reduplication of chemical molecules is discussed according to the views of Haldane and others (33).

Cleavage does not result in increase in mass of the cell. This remains for later processes. Cleavage, per se, is not a cause of differentiation; differentiation occurs without cleavage.

Malignancies increase in mass because their protoplasmic centers of activity, namely, cells, increase in number, but as far as anatomical differences characteristic of malignancy are concerned, none have been found in spite of numerous studies; that is to say, when "healthy" cancer cells in active division have been compared with normal cells in the same stage, no specific differences have been found (34). Since malignancy is manifested by failure of proper differentiation and complete lack of organization, it is stated, because cell multiplication is not basically concerned in differentiation, that malignancy cannot be a disease in which cell multiplication is anything characteristic.

Furthermore, cell division activity in tumors proceeds in waves as it does in normal growth. There are peaks and valleys. This is mentioned because large numbers of cells in mitosis are signals sometimes falsely interpreted as a criterion of high malignancy and, conversely, small numbers are indicators of low malignancy. Obviously how many cells are in mitosis in a given specimen depends on when the surgeon happened to remove the tumor and the instant of fixation. Did the students ever count the number of mitoses in a piece of rapidly grow-

ing normal growth? If so, they would find many; indeed it would be a matter of surprise if they could not find them. Experiments to determine the timing relations of the waves of mitosis yielded equivocal results, but apparently at least three peaks occur every 24 hours. Irradiation is probably more effective during mitosis and attempts are made to deliver dosages at such intervals as may correspond to the peak times of mitosis. Colchicine halts mitosis temporarily and this drug is being administered by some radiologists about four hours before a contemplated treatment. No definite data are available as yet, but encouraging reports have been heard.

The rate of multiplication of cells in malignancies is discussed in relation to growth rates in general. Numerous figures are given of growth rates of various kinds of cells, tissues and organisms. Methods of measuring growth rates are rehearsed. Distinctions between absolute and relative increments, between true growth rates and mean growth rates, between differential growth, etc., are discussed (35). Finally, in relation to malignancy it is emphasized that, fortunately, malignant tumors grow at rates very much slower than the rates which can be exhibited by developing embryos, regeneration, repair and other normal incremental growth processes.

2. *Differentiation*

Differentiation of a cell, as stated, involves a change in its chemical make-up which is reflected in its anatomic appearance. From more general, it becomes a more special kind of cell.

An important generalization in regard to the ability of cells to differentiate, is that they are potentially able to do more than they actually do. Their developmental possibilities are greater than their developmental fates. Within cells are possibilities; the closer to the fertilized ovum, the more the possibilities. As the possibilities are brought to actualities the number of possibilities diminishes. Chemically, undifferentiated cells in general have within them more "open bonds," that is, unsaturated electron pairs, than differentiated cells. Since the number of combinations possible with the materials supplied to growing cells is almost infinite, it is small wonder that their potence which is an expression of their chemical and physical possibilities is greater than their realization. Realization means that one particular possibility of the many has been picked out for action. Unfortunately there are no ways at present of measuring quantitatively the possibilities of differentiation nor yet even degrees of the processes. Re-

course must be had in the latter case to judgment based on experience which in turn takes note of appearance of the cell constituents, ratio of nucleus to cytoplasm, position of the cell in relation to others within the tissue, etc. As far as measuring qualitative potencies of an undifferentiated cell is concerned, there are no means available except to allow the cell to develop and see what happens. It is pointed out that when histologists attach the suffix "blast" or some similar one to a cell with a connotation of its being a potential forefather of cells of a definite kind, they usually are but guessing. For many years, pathologists have labored at the exact derivation of the cells of tumors; the very nomenclature and expressions used to describe many tumors are heritages of this work. A squamous cell carcinoma is said to "arise from squamous cells." This method of expression is incorrect in the light of our present knowledge of general developmental biology. It is quite certain that when a cell reaches a definite degree of differentiation it can no longer divide. This is part of the general statement that differentiation and division are antagonistic. All tumors must arise (and continue to increase in mass) from division-capable cells. Only such cells are capable of division which have not completed their differentiation, indeed which have not arrived at certain degrees of differentiation. Therefore all tumor cells must arise from incompletely differentiated cells. But if their differentiation has not been completed or determined, they can do more than they actually accomplish. Therefore we cannot say that a squamous cell carcinoma arises from squamous cells, for squamous cells are well on the road to complete differentiation and cannot divide. Squamous cell carcinomas therefore arise from partially differentiated cells which differentiate out as far as imitating squamous cells, and therefore they are called "squamous cell carcinomas." It is a wonder that no one has as yet called them "squamoid cells"!! This point of view, somewhat different from that ordinarily expressed, seems more in harmony with the present-day facts of experimental embryology. Furthermore it helps account for the varied appearances of different parts of one and the same tumor if it is agreed that disturbance of the differentiation process is one of the characteristics of malignancy.

3. *Organization*

Development is epigenetic and not pre-formed. One part in developing brings to bear its influence upon the parts succeeding. In the fertilized ovum are the potentialities for the completed organism,

not the actual parts in miniature. This can be visualized chemically as guiding enzymes determining unions of the various bonds. From the environment the organism incorporates building blocks and synthesizes them into its own specific substance. Since malignancy does not organize at all, in the sense in which this word has been defined, it follows that there is abnormal synthesis of substance and/or synthesis of abnormal substance. There is a world of difference between these two activities and the students are asked to think them through. The chemical basis of life is protein and therefore the building blocks of protein are the most important constituents which the environment can provide. Philosophically, living substance is a highly improbable state of affairs, and since ordinarily, highly improbable aggregations of matter are unstable, living substances are therefore temporary. Among other arguments, the following will serve to illustrate the point. Among the amino acids, as well as other compounds, living substance as a general rule uses only one of many possible isomers. Ofttimes it is the levo-rotatory form, occasionally the dextro-rotatory form. These substances are, of course, synthesized from simpler ones, many of them by plants but some also by animals. In the laboratory the chemist in synthesizing an amino acid usually has a fifty-fifty chance of making either a dextro-rotatory or a levo-rotatory form. In fact in his preparations he usually has a mixture of approximately equal parts of both. Living substance, on the other hand, performs the highly improbable feat of preparing (and using) only one of the isomers. Therefore life is a highly improbable state of matter and consequently temporary (36).

The work of Kögl has received destructive criticism from many workers (37). Nevertheless it has not been entirely contradicted. Whatever the outcome of these experiments may be, it still remains a theoretic and philosophic possibility. Kögl and his co-workers present evidence that in malignancy there is a larger percentage of the "unnatural" forms of amino acids than in normal tissue. The fact that malignancy is anatomically different from normal tissue, that it behaves differently physiologically is sufficient theoretic evidence that there is a difference in composition and chemical growth processes.

Development being epigenetic, i.e., step by step, one stage developing in relation to the immediately preceding stages, receives abundant confirmation and proof in the "organizer" phenomenon as elaborated by Spemann, his co-workers and many others. The principle of "pro-

gressive organization," to use Spemann's appropriate expression, is exemplified by many examples (38).

The student's attention is called to the innumerable questions which can be asked in relation to these phenomena and malignancy, for it is emphasized again that malignancies do not organize, they do not obey the impulses coming from the organism as a whole and which normally initiate, determine, control and organize all other growth such as regeneration, repair, differential growth, etc.

The questions which can be composed in relation to organization and non-organization, it is emphasized to the students, are among the crucial ones in malignancy. Much more time probably should be given to them but other affairs require discussion. Time is taken for mere mention of a few of the factors which control organization. In the beginning it is stated that the few cells of the developing embryo are in close enough contact with each other to be guided mutually in their respective proper paths. Later diffusion and physical contact no longer suffice and there are developed such organizer helps as the blood vessels and blood, the nerves, the hormones as coming from chemical differentiation of cells; fields; function, as in functional differentiation; gradients are developed, growth centers carry on (39). Of these, because of their present clinical importance, hormones are singled out for more thorough discussion. The concept of chemical differentiation also receives more attention. The opening question is of course, "What relation do these have to malignancy?"

4. *Hormones*

It is stated that hormones have been evolved as far as their activities in growth and development are concerned for guiding differential growth. They play upon the potencies of cells, adding nothing and subtracting nothing from their already present complement. As examples, the surge of activity of the gonads just before and during puberty causes the cells of certain organs to express more of the potencies which are already present in them leading to a change in the differential contours of the body. As a pathological example, the change in differential contour, namely, the large head, hands, and feet of acromegely are mentioned. The axolotl lives in water, breathing by way of gills but tiny indifferent fringes on each side of its head develop into lungs under the influence of the hormone from the thyroid gland. The fringes expand their already present potencies and

develop into air breathing organs. The axolotl then drowns if held under water (39).

Direct experiments with hormones on the malignancy problem are then quoted in considerable detail. Addition and subtraction experiments have been done. When various hormones, especially the sex hormones, are presented to animals, there follow a series of changes, most of which can be classified as metaplasia. Thus squamous cells replace the cylindrical cells lining the prostate glands. Squamous cells replace the lining of the uterus. Male mice are caused to develop breasts as large and extensive as those of their sisters but never as yet, of and by themselves have hormones been observed to produce cancer directly (Loeb). In Lacassagne's experiments, in those of Allen and his co-workers, the incidence of carcinoma of the breast in male mice has been increased from practically zero to almost as high as that in their sisters by very early injection of female sex hormones. Variations by others of the experimental approach consist of transplantations, etc., but in all cases thus far investigated it seems that the increased incidence of cancer in male mice occurs only in strains in which cancer potentially of that organ had been inbred. Further details are given under genetics and many more when clinical breast and uterine cancers are presented. These experiments, it is stated, follow in logical succession, those done many years ago by Leo Loeb in which he found that castration of female mice from a cancer strain resulted in fewer cancers of the breast. Removal of the ovaries resulted in less volume of breast tissue. In the case of injections of hormones, transplantations of ovaries, etc., into male mice the result was as much breast tissue as their sisters ordinarily enjoyed.

The amount of hormones ordinarily given clinically is much less than experimentally given to mice. Whether or not they predispose to the development of carcinoma in women is undecided. We cannot as yet determine by any methods which of a strain of animals or human beings is susceptible to malignancy. If hormones cause cellular activity in breasts or uteri, there is more statistical chance of cells undergoing malignant change when a thousand are active than if only ten are active, assuming there is an hereditary taint in the background (40).

5. *Chemical Differentiation*

Chemical differentiation is illustrated by a number of examples, several of which will be given here. The cells of certain tumors de-

velop within them tiny chemical laboratories which manufacture specific products. When these products can be identified their presence acts not only as a diagnostic measure but also as a guide to therapeusis. Among such tumors wherein chemical differentiation provides these telltales for analysis are the embryonal adeno-carcinomas of the testis, certain adrenal cortical tumors and certain ovarian tumors. Indeed the fruition of chemical differentiation in tumor cells may be identified by the actual physiological effects produced within a patient. Thus, for example, a patient long past menopause, begins to bleed from the uterus and develops a periductal fibroma of the breast. The possible causes are rejuvenation of the ovaries or a tumor producing hormones. Practically always it is a granulosa cell tumor of the ovary (41). More discussion and examples of these interesting problems are given when patients with breast, uterine, and endocrine gland tumors are presented.

6. *Genetics* (42)

About one-third of the classes had not had genetics in their college courses and those that had admitted haziness. Therefore, a few of the classic experiments in genetics are rehearsed and general principles expounded.

Variation is due to (*a*) environment, (*b*) hereditary mixtures, and (*c*) mutations. It is emphasized that the question of which is more important, heredity or environment, has as little meaning as the question of which is more important in a ball game, the ball or the bat. The import of this question is put in another form. Upon any given characteristic what influence has environment and what influence has heredity?

To illustrate variations due to environment, Johansen's experiments with beans are rehearsed. Then the famous diagrams of crosses between red and white four o'clocks are introduced. Examples of mutation are mentioned, along with the story of the production of mutation by x-rays (43).

Mendel's experiments and laws are recalled. There is no such thing as "mixed" blood. Chromosomes and genes do not merge, just as atoms do not merge in chemical compounds, but are shifted around to appear and reappear. Separate gene pairs are shuffled and dealt out independently of each other. Dominance and recessiveness are illustrated by examples such as that of waltzing mice. It is emphasized that a recessive gene is never destroyed but remains in hybrids even

though its presence cannot be known until the right combination occurs as, for example, in deaf mutism and cyclops. Various examples of gene effects are given such as color of eyes, shape of ear lobes, albinism, etc. Lethal genes are mentioned with the example of the crossing of the yellow and gray mouse. The studies of C. B. Davenport are given as examples of the effect of multiple genes in the crossing of pure white and pure colored stocks. The mapping of genes is mentioned. It is emphasized that the numerical proofs of Mendel's laws are based on random fertilization, but that interference occurs from various environmental factors, that there is a higher motility of some zygotes, that chromosomes occasionally stick together, etc.

While many inherited characteristics are transmitted by way of chromosomes and genes, nevertheless there is also a non-Mendelian inheritance as exemplified particularly in certain plants wherein the cytoplasm also carries factors of inheritance. Mrs. Harvey's non-nucleated sea urchins are mentioned, and in passing, the possibility of cytoplasmic transmission of the predisposition to special kinds of cancer in certain strains of mice.

Superstitions about heredity such as telegony, maternal impressions, etc., are mentioned. In all of these discussions there is constantly reiterated the necessity for obtaining as pure lines as possible for experiment. This is done by inbreeding and back crossing. Brother to sister, mother to son, father to daughter matings, etc., are used. It takes at least 20 generations of this kind to begin a "pure" strain. It is a necessity to have these for experiments not only in genetics but also for the evaluation of environmental factors. These latter are varied in a controlled way against a background of as uniform an heredity as possible. Inbreeding per se is not harmful to a stock, but in animals, the undesirable offspring can be eliminated. The taboo against human inbreeding arose from the fact that such matings bring out the skeletons in the closet in the shape of many undesirable recessives and those inheriting them and manifesting their results cannot be eliminated.

Turning to the inheritance of cancer, it is stated that cancer itself is not inherited but the potentiality for its formation may be. As a result of many years' experiments, the following may be stated at the present time. The predisposition to cancer can be inbred in animals. It is not manifested for cancer in general but rather for cancer of a specific organ or site. It is not transmitted as a simple dominant nor as an ordinary recessive. There is cytoplasmic influence in certain

breast tumors and possibly in others. Numerical treatment by ordinary Mendelian laws is difficult and indecisive.

There is then brought up for discussion the influence in human cancer of heredity and chronic irritation. That cancer is from an inherited predisposition has been denied. It has been said that it must be present in one or both parents otherwise it is not hereditary, or if it is to be inherited, it should be present before, at, or shortly after birth. These statements are the result of complete misconceptions of the mechanisms of inheritance. Other objections to the belief in the hereditary nature of tumor predisposition are emotional. People do not like to believe it, but the question is not "is it a likable belief, but is it true?" The chronic irritation doctrine has occupied excessive prominence probably because more can be done about chronic irritation than about heredity. Heredity and irritation are not mutually exclusive. It is pointed out that in human cancer only the faintest beginnings have been made in collecting the necessary information for final judgment. The animal breeder can breed at will without taking the romance out of marriage. He may mate brother to sister, father to daughter; he may breed constantly, whereas in human affairs too many offspring may need appropriation of public funds for their upbringings. Finally the animal breeder may terminate the lives of his subjects at any time. The student of human inheritance, on the other hand, must wait for appropriate matings. When appropriate, the couples involved may not breed at all, or they may produce only one or two offspring when 16 or 20 may be needed, or the offspring may not be of the right sex.

In spite of all these difficulties it is pointed out that progress has been made. In many diseases the mode of inheritance is known and the mathematical chances can be calculated. This is not true of cancer but there are many case records of mother and three daughters with carcinoma of the breast, mother and four children with carcinoma of the rectum, three brothers with carcinoma of the bladder. Numerous case histories of twins are on record in which at approximately the same age the twins develop cancers of the same organs. The work of Madge T. Macklin (44) is quoted. For instance, in the case of the mother and four children with rectal carcinoma, if chance alone had produced this occurrence there must have been living on earth 4,668 billion persons. Such cases are by no means unique or isolated. Evidently there is such a thing as the inheritance of a predisposition to the development of cancer in human beings as well as in animals.

That it does not always appear is not to be wondered at, for there may have been only one child, the individual may have died before the development of the cancer, it may not have been of the right sex and the right stimulus may not have been received. Apparently there are stronger and weaker predispositions. Of course, such figures and considerations while they prove a greater than chance incidence give us no data on the next step, the "how" of the inheritance.

The question is brought up "Can cancer occur or be produced in an animal that has no hereditary predisposition to the development of the disease?" The answer to this question cannot be given as yet but at present it is apparently true that in experiment there is an hereditary background when cancer is developed or produced by any of the carcinogens. Other facts and theories are mentioned such as the part of extra-chromosomal transmission in mouse mammary gland tumors, the effect of foster nursing in influencing the incidence of mammary tumors in mice, the inheritance of pulmonary tumors, of leukemia and kindred subjects. Naturally again, opportunity for rehearsal and more extended discussion arises when patients are presented (45).

The final studies of genetics concern sex determination. McClung's definition of sex is used—"it is the dual expression of a common series of characters unequally and reciprocally developed in the individual." Another definition is: "sex is a result of common characteristics expressed as different totals." It is emphasized that there is a little bit of man in every woman and a little bit of woman in every man, that there are gradations of this "littleness" from the real he-man on the one side and the real she-woman on the other to a center in which the two characteristics are nearly equally distributed!!

The reasons for dwelling on sex determination are the facts that abnormal growths such as gynecomastia occur, that the sex organs are subject to numerous tumors some of which produce changes in the sex characteristics.

Sex determination, it is stated, is due to chromosomes but is of three types: (*a*) due to genes almostly entirely as in insects, (*b*) due to genes and gonads as in mammals, and (*c*) due to genes and environmental changes as in oysters, crepidulae, etc. The role of "*X*" and "*Y*" chromosomes is discussed. The fact that there is a higher metabolic rate in males is mentioned as perhaps determining whether the indifferent cells in the early kidney region are to be ovaries or testes. Gynanders and freemartins are mentioned. Sex reversal is discussed

with illustrations starting with the famous rooster of Basle which started life as a hen, became a crowing cock, was arrested, tried for witchcraft and solemnly burned at the stake.

"A whistling maid and crowing hen
Are shunned alike by God and men"

It is pointed out that sex reversal in female birds is more readily accomplished because they have an ovo-testis, usually on the right side (46).

Examples of sex-linked inherited defects and diseases are given such as hemophilia, night blindness and myopia, optic atrophy, etc.

7. *Regeneration As a Function of Cell Potency* (47)

Regeneration is post embryonic growth and development. It has no part in the primary formation of the individual but appears after completion of morphogenesis.

Regeneration, like embryonic growth, is progressive, i.e., it grows from the simpler to the more complex. It is secondary and not a part of the primary development.

The ability to regenerate is widespread in all species and in many parts of the body. Certain taxonomic groups, for example, regenerate whole limbs. Triton, crepidulae, etc., regenerate astonishingly organized parts such as eyes. The ability of parts of human beings to regenerate is also well marked and widespread. Human beings cannot, of course, regenerate whole arms or legs, but the liver can regenerate at least one-third of its mass within two weeks as exemplified, for example, following the destruction by yellow fever. The thyroid gland regenerates. The endometrium and parts of the breast regenerate rhythmically.

In this respect, therefore, regeneration is similar to cancer, the potentialities for which are widespread, practically universal, and in any and all tissues.

Regeneration is not an adaptation but part of the primary potence of an organism. The power of regeneration and the frequency of loss of parts do not always correspond, which is also true of cancer. Regenerates may appear without previous loss of a part, even without noticeable injury, as does cancer.

In general, the power of regeneration is inversely proportional to the degree of organization of an organism and its stage of development. Regeneration shows that the whole of potency is not accounted

for in ontogeny. While there is variation in different species even those organisms developing from so-called mosaic eggs retain the ability to regenerate (48).

As a supplementary performance, regeneration is limited in some organisms by special processes which diminish or check the potency of separate parts during ontogeny. The students are told that more knowledge of the physics and chemistry of these checks might be useful in devising checks on the growth of cancer.

The excess potency is so great in many instances that there seems no limit to regeneration and there can be multiple regeneration of regenerates. To some extent, conditions depend on the wound surface and to a large extent on where separation of the old part has occurred. In regeneration, "like does not always beget like." The three germ layers are not sacred; muscle may come from presumptive epiderm, etc. Metaplasia is common. For instance, the lens of the eye of triton may be regenerated from the iris, a foreign source. Normally it comes from the epidermis of the head. Heteromorphosis occurs. A different organ may be regenerated; for instance, a mouth at the anal region, antenna instead of claws, and vice versa. Interesting though these processes are and important though it may be to accent what is known of many of the conditions affecting the heteromorphosis and metaplasia of parts, the students are hurried to the following considerations. Whence arise regenerates? Immediately after separation of the old part, whether artificially or by autotomy, i.e., spontaneous amputation by strong muscle action, a blastema appears. Cells undergo rapid multiplication and as the oncoming cells are produced, differentiation begins in some of them. As a concrete example, studies of the regeneration of the hermit crab claw are cited in detail and illustrated with photographs and photomicrographs (49). It is pointed out that while de-differentiation of certain cells may be possible, nevertheless it is extremely difficult to follow one particular cell and watch its progress. That tissues are de-differentiated there can be no doubt, that is to say, in place of the part lost there appears an undifferentiated mass of cells or finally a tissue less specialized, but whether it came from cells in the stump which de-differentiated, then divided and proceeded to re-differentiate again, or whether it came from cells which never differentiated, is an open question. It is merely emphasized that, certainly, there is a stage in the differentiation of the cell beyond which it cannot return to its original condition and that in all probability most of the new growth comes from undifferen-

tiated cells which are present in practically all situations of the bodies of any and all animals. These cells we call "spare parts," as indicated before and teach that the bulk of all post-embryonic growth occurs from these cells. Spare parts supply the necessary centers for repair, regeneration, hypertrophy, hyperplasia, response to infection, and—we also teach—tumor formation (50).

Since undifferentiated cells contain more potencies than they use, it follows that the various kinds of cells necessary for the completed regenerate arise by the determination of, and expression of one or the other of the various potencies in these cells.

Cancer does have a predilection for arising in places where there is constant cell activity for replacement, repair, regeneration, etc., such as the breast, the uterus, the skin, the vermilion border of the lips, etc. We do not know as much about replacement activities in the esophagus, stomach, rectum, and other situations but there can be no doubt that replacement is needed as cells grow old and desquamate.

It is emphasized further that the phenomena of regeneration are excellent study material for cell potencies and behavior, growth rates, cell chemistry and physics, and, quite important, comparisons with abnormal growth and development. The crab claw regenerating material is an example of the latter. In a number of instances, injuries occurred to the tender new regenerates which disturbed their orderly organization. Cells on the surface became epithelial, underwent chemical differentiation which allowed material for the new shell, chitin, to be formed. This shell acting as a barrier (organizer) kept the proliferation within it in bounds. When the shell was broken by injury, cells streamed through and formed small bumps and lumps over the surface. Did these cells continue to proliferate as they would if they were malignant? No, they did not! They gradually differentiated, somewhat irregularly it is true, but nevertheless differentiated, gathered themselves together in masses and stopped proliferating. The ability to differentiate contained within them was not destroyed or injured and they did the best they could under the changed environment and without all the normal organizing influences. Certainly they did not turn malignant. The same was true in several specimens in which a second regenerate formed within the primary one as it was developing (51).

These, and the skin specimens previously described (52), are correlated with many more examples to lead to the following generalizations:

Malignancy is due to a change in the original make-up of cells which involves particularly their potencies of differentiation and organization. All environmental factors which "cause" cancer must finally be correlated with intracellular chemical processes whether they be carcinogens, irradiation, viruses, chronic irritation, or what not.

This, it is stated, is the famous "somatic mutation theory of cancer." But hastily indeed is semantics involved. What is the meaning of "somatic mutation"? To me, I tell the students, it means that the internal make-up of certain somatic (not germ) cells which was bequeathed to them from their ancestors is changed chemically and physically. This statement at least concentrates their attention on cellular chemistry and physics and I hope makes some of them want to help tackle this fundamental and enormously difficult problem. In their armchairs, by the fireside at home they may marvel at the wonders of protoplasm, appreciate more deeply Harvey's statement that the "efficient cause of the chick is hard to be found out" and speculate on the treasures to be unearthed by such studies.

8. *Transplantation, Explantation, and Interplantation*

A very important method of studying growth and development consists of removing individual parts of an embryo, or tissue, or organs from a younger or older animal, thus removing them from their environment and placing them in another, perhaps more controlled, one. Transplantation is, of course, of much practical value in surgery, and in cancer surgery especially it is of the greatest use in repairing defects resulting from surgical removal of tissues, or following the removal of heavily irradiated parts which do not heal. This is an opportunity to call the students' attention to the following: (*a*) Reconstructive surgery has made great advances during the last years. (*b*) To "cure" a cancer it is necessary to remove it in toto. (*c*) The defects remaining after sufficiently extensive removal of tissue to cure cancers are never as disfiguring or disagreeable as those produced by the cancer itself. (*d*) Defects can be repaired surprisingly well if the cancer is completely removed but not if any cancer remains.

Transplantation is distinguished as autoplastic (one part of an animal to another of the same animal); homeoplastic (part from one animal transplanted into another of the same species); and heteroplastic (transplant and host of different species or even genera). By zenoplastic transplantation is meant transplant to host of widely different species.

Successful transplantation implies a definite degree of genetic or species relationship between donor and host varying somewhat with age and species, degree of organization of the part, and sufficient blood supply. Many details are given of results in this field. Specified bits of embryos have been transplanted to others of the same or nearly related species. Parts have been exchanged. Presumptive fore and hind limbs of triton have been amputated and their positions reversed by transplantation. Presumptive epithelium transplanted to other regions has turned into bone and vice versa! Eyes have replaced antennae and vice versa, etc. Several generalizations are then rehearsed. (*a*) Potency is greater than realization. The three germ layers should no longer be considered sacred. (*b*) As potency is used, it is diminished. (*c*) The effects of various chemical compounds and mixtures are told, to be repeated later under effect of environment on growth and development. (*d*) Malignancy has never been produced by mere transplantation into different regions.

The students are told that an important distinction is to be made between the transplantation of normal tissue and that of cancer. Both "take," but malignant cells continue to multiply, probably because they do not differentiate normally nor attempt to organize at all!! Normal tissue, on the other hand, if the transplantation is successful tends to differentiate and even organize to certain degrees. Thus ovaries, thyroid, and a host of other tissues can be transplanted and produce the specific secretions under these circumstances which they do in their normal positions. Merely a "take" therefore is not a criterion of the malignancy of a transplant. It should continue to grow indefinitely, i.e., until the host dies. The percentages of "takes" vary, however; thus a particular carcinoma of the breast of a mouse when transplanted under standard conditions of quantity and situation to another mouse will "take," say on the average, 50 per cent of the times, whereas the transplants of certain sarcomas "take" in practically 100 per cent of trials. Not only this but host strain differences also appear and it is possible, the students are told, to breed mice toward higher susceptibility or toward lower susceptibility to a given transplantable tumor. When a particular family of animals has been brought by inbreeding to a point of high susceptibility to transplants of a certain tumor, this high susceptibility will not necessarily apply to other tumors (52).

Much has been learned about cell behavior from studies of transplanted tumors, but it must always be remembered that in such cases

the tumors do not arise from the tissues of the host but from some other individual. The host acts as a sort of glorified tissue culture slide or tube. Probably, human tumors are transplantable to others of the same species.

Students are told that, in general, experiments on therapy are less reliable when transplanted tumors are used as test objects than spontaneous tumors. Many are so easily "cured" that a cancer worker dismissed a dream-inspired layman who "cured" the transplanted tumors of a few mice given to him with a decoction of dandelion leaves, with the remark, "Oh, I can cure those with Ivory soap."

Explanation means the removal of a bit of tissue from an animal's body and its seeding into culture media in a tube or flask or on a cover slip inverted over a hollow ground slide, or by several other methods.

Tissue culture has become very familiar to many people and so, in relation to malignancy, a few remarks may be made. The tissues of all animals which have been subjected to experiment have been grown in culture. Malignant tumors, human as well as those from animal sources, have also been cultivated. The differences, as displayed in tissue culture, between the growth of malignant cells and their corresponding normal ones, for instance, spindle cell sarcoma and connective tissue, are definite though subtle, and require some experience for recognition. Malignant cells grow more rapidly and abundantly, display more variable patterns, liquify clots in culture media more rapidly than normal cells, produce more acid and, in subcutaneous transplants placed into a suitable animal, they "take" in a much larger percentage of cases than do normal cells. When they "take" they continue to grow and, of course, kill the host. The characteristics of malignancy are retained by these cells during many subcultures and thus justify the idea that they are a new race of cells, to use the expression of Warren Lewis (53). They do not return to normal cells. On the other hand, there have been no methods discovered which cause normal cells grown in tissue culture, to turn into malignant ones.

All in all, while much has been learned from tissue cultures, nevertheless, if malignancy is primarily a disease of organization, tissue cultures cannot be expected to yield information on this point. They are not organisms and as such, while attempts at differentiation do occur and even organogenesis, e.g., as Stern has shown with the testis of Drosophila (54), the growth is not of a Whole.

Interplantation has yielded results of late of considerable interest

in oncology. This procedure consists of removing a bit of tissue from one organism and transplanting it into a body cavity of another animal. Species differences do not play such an important rôle in this type of experiment. Human tissues have been grown within the eye cavities of rabbits. The mass of growing tissue imitates, in a more or less recognizable manner, the pattern which it developed in the animal from which it arose. Thus considerable information has been obtained about this phase of the subject. The method has just been begun and its further exploitation should yield considerable knowledge (55).

Attempts at plant tissue culture have been unsuccessful until the last few years when White succeeded in growing in this wise an isolated plant organ, the root (56). Perhaps perfection of this method in the future will yield information from this source.

9. *Carcinogens*

The story is told of chimney sweeps' cancer and soot (Percival Potts, 1775). There is clinical recognition that other agents than soots produce cancer and most of them by way of "chronic irritation." Attempts to reproduce cancer in animals by rubbing them with soot and tar were made quite early but very definite results were obtained by Yamagiwa and Itchikawa in 1915. Rabbit ears were tarred for many months and finally by way of chronic inflammation, overgrowth, and skin papillomas there appeared true squamous cell carcinomas. In Woglom's publication of 1926, 290 references are given (57). From 1926 to 1932 some 400 additional are recorded. Other simple chemicals such as arsenic and zinc chloride were found to produce carcinoma but not with any consistency.

From all the publications on the use of tar, certain generalizations are told to the students. (*a*) Application produces malignancy in certain laboratory animals such as rabbits, mice and rats. On the other hand, guinea pigs are extremely refractive and while inflammation and even papilloma can be produced, nevertheless it is in an exceptional case when carcinoma appears in these animals. (*b*) It is not known at present whether the production of cancer is due to purely local conditions or to constitutional factors or both. It appeared, however, in later years when the breeding of "pure" strains of mice had been perfected that certain strains are more susceptible to a given tar than others. This is particularly true of lung tumors which develop in tar-painted mice, but also appears to be true for those that

develop in the skin. (*c*) Neither sex nor age is important. (*d*) The time factor is the same for young or old animals. From six months to fifteen are required in most instances. (*e*) Some tars are more carcinogenic than others. This is apparently related to boiling points for those of higher boiling points are more active. (*f*) There is always a latent period. (*g*) Tars start malignancy but they are unnecessary for its continuance. (*h*) Certain active tars need not be applied until the carcinoma actually begins. Applications may be interrupted after several months and carcinoma develops, say, at six or nine months later. (*i*) Tars produce both carcinoma and sarcoma and from all classes of tissue, illustrating again the general principle that malignancy is a universal cell potency. (*j*) The transformation is sudden. This statement requires amplification and will incite differences of opinion. It can be allied to the famous controversy concerning the word "precancerous." The students are given certain considerations about this and then are asked to think them through, meanwhile being alert for new facts as they appear.

When a lesion is called "pre-cancerous" it is a prophecy. When this diagnosis is made clinically, the lesion can be watched to see if it becomes carcinoma as time progresses. If it does and if it is certain that it was not cancerous in its beginning, the diagnosis "pre-cancer" is justified. But *early* cancer is a diagnosis to be made not clinically but by the microscope, and it is quite in order to ask whether a microscopic section of the lesion called pre-cancerous was made when that diagnosis was issued. If it was not, there is room for reasonable doubt. If it was, then the disturbance of biopsy, etc., is added to complicate the picture (57). Apart from the fact that no good doctor will allow a pre-cancerous lesion (assuming it is such a lesion) to progress into a cancer under his eye, if the microscopic diagnosis is considered unimportant, much of our propaganda for early diagnosis is brought to nought. The students are told over and over not to trust to clinical judgment in the diagnosis of early cancer but to rely on properly obtained biopsy material. They are told over and over that the clinical diagnosis of cancer when it can be made from across the room is of little comfort to the patient, relatives, and himself. If an individual must have cancer, it should be diagnosed at a time when no clinician, no matter how astute, can establish that diagnosis. In addition, attention of the students is called to the pictures produced by various carcinogens in certain animals, particularly guinea pigs. They are told that of a series of 50 guinea pigs, the skins of

which were rubbed three times a week for five years, that is, from the time they were weaned until they died of tottery old age, not one developed cancer. But everyone showed any and all of the lesions which have been described (enthusiastically by many) as pre-cancerous. Since the guinea pigs did not develop cancer these lesions are not pre-cancerous.

As a modification adapted more to clinical than scientific purposes, the students are told that it is safer to err on the side which regards lesions as pre-cancerous than on the other side which allows lesions to become cancerous under their noses. It is emphasized over and over that if suspicious lesions are removed and proved to be non-cancerous, certainly these lesions which are safely in formaldehyde, or have been fixed, cut and stained and are on a microscopic slide cannot produce further trouble for the patient.

To return to carcinogens, they are told the story of the success of the London Free Cancer Hospital workers,—Kennaway, Cook, Mayneord, Hieger, and others (58). These workers, proceeding to isolate the active principle of tar, studied its fluorescent spectra. They found that there are characteristic ones in carcinogenic tars and oils. Acting on this information, they produced 1:2:5:6 dibenzanthracene which proved to be carcinogenic. Since that time many other carcinogens have been synthesized, and one, namely 3:4 benz-pyrene, has been isolated from coal tar (59). Similar findings are recorded for these substances as for the tars themselves. There is no obvious chemical relationship between them. Some are not hydrocarbons and some contain nitrogen. Yoshida in Japan (60), feeding rats ortho-amido-toluol to discover its effect on the thyroid gland, found that carcinoma of the liver appeared in a number of his animals after prolonged feeding. This work can be correlated with that of Fisher on scarlet-*R* which has ortho-amido-azo-toluol in it. Other workers, in confirming these results, have found, as Yoshida did, that diet plays an important part in the production of carcinoma of the liver with this compound. More data are needed before this question can be settled, but emphasis is laid on this compound because it is fed by mouth and because it picks out a specific organ on which to exert its effects. The biotin angle is discussed. Several other compounds are mentioned, such as styryl which is a quinoline derivative; tri-phenyl-benzene and tetra-phenol-methan have produced cancer. Some of the compounds are much more active than others in producing a higher percentage of cancers and/or in a shorter length of time.

The greatest interest, the students are told, lies in two directions: (*a*) to discover what chemical changes are produced inside of cells to which these compounds have been applied, and (*b*) to try to discover compounds which conceivably could be produced naturally by cells. In the case of the former, it is suggested that groups, compounds, and configurations which control organization of cells and tissues are the crux because cancer does not organize in the sense of that word as given before. Needless to say, knowledge of these factors in normal organization is meager indeed and must be known before any tales can be told about them. As far as the latter is concerned, normal intracellular metabolism is the key. For instance, chemical relationships of some of these compounds to the sex hormones are discussed. Some of them have actual estrogenic properties. Theoretically, there are chemical relationships as to cholesterol, a constituent of all cells. Roffo's work is cited and the possible relationships to parts subjected to sunburn via cholesterol and allied compounds. It is stated that carcinomas of the skin are more frequent in farmers, fishermen, sailors and others whose occupations keep hands and faces particularly exposed to sunlight.

Methylcholanthrene is a degradation product of desoxycholic acid and cholic acid which are constituents of the bile. But much more work is required before all these points can be clarified. As far as the effect of any or all of them upon intra-cellular chemistry is concerned, not one bit of settled knowledge is at hand at the present time. A curious fact is also mentioned, namely, that mustard gas which is diethyl sulfide protects against the carcinogenicity of some of these compounds, and so does the sulfhydryl group as present in several compounds when applied before or simultaneously with the carcinogens. Cystine given by mouth helps protect rats' livers from butter yellow cancer as it does skins from cancer by methylcholanthrene (61).

As far as methods are concerned, the students are told that pure strains of animals should be used and two questions answered—How many cancers per standard time, and how early are they produced? The chemical may be rubbed into the skins or be inserted into pellets of innocuous substance which are buried in the tissues or they may be administered orally, or via the respiratory tract.

Within the last year or two progress has been made in studying the metabolism of several of the compounds when given orally or sub-

cutaneously. For instance, 1:2:5:6-dibenzanthracene remains as such for long lengths of time in the tissue. More data are awaited.

It is pointed out also that there is a general systemic effect. There are changes especially in the lymphoid system and in the liver similar to those which result from general exposure of the animal to x-rays. Ofttimes there is lowered resistance to the transplantation of tumors. They occasionally increase the natural tendency to develop cancer in predisposed organs, for instance, the lungs. Sometimes they elicit cancer in other parts than where applied.

The relation of the compounds to "chronic irritation" is mentioned. Most of them are irritants, some violently so. But others are not, e.g., styryl 430 produces no irritation whereas its non-carcinogenic isomer, styryl 245, does. Corresponding to the fact that even a few applications and then cessation are sometimes followed long after by cancer in the rubbed places, histologically such places show almost immediate disorientation especially of the basal layer of the skin from which proliferation occurs. There follows disturbances of keratinization, the surface cells adhere long after they should have desquamated and cell division rate is stepped up. These, it is pointed out, are again evidence of disturbed differentiation and organization of the cells.

Immediate clinical considerations include analogies to aniline cancer of the bladder, the detailed story of which is told of how the tumors develop after eight, ten or more years of exposure, or even after but a short exposure years before. Every class thus far has had presented to them at least one worker in tarred rope with cancer of the finger tips where splinters of the rope have carried tar into the skin. Several patients have presented themselves with cancer of the skin developing in the scars of burns from splashed hot tar. Arsenic cancer is mentioned as well as several other well known types.

10. *Infections, Infestations, and Viruses as Related to Cancer*

Attempts to link micro-organisms and cancer date from early time. The rise of bacteriology as a science naturally stimulated interest in a possible relationship and at some time or another almost every known organism has been accused of causing cancer. "Cancer houses" are described, "cancer communities" and "contact" infection were accepted as facts by numerous individuals. The students are told that while certain organisms have been found so closely associated as to be regarded as "causes" of cancer, they have always been very

special ones and in very special situations. Fibiger's nematodes and cancer of the stomach in rats are mentioned. Tapeworm cysts in the livers of rats are associated with sarcoma of that organ. The studies of Wood and his associates, Bullock and Dunning, are described. Bilharzia in man is associated with carcinoma of the bladder.

In 1911, Rous described fowl sarcoma as being transmissible by factors separable from the cells (62). Succeeding studies of these, as well as other tumors of like nature, are detailed.

Work with the Shope rabbit virus is described in which papillomatous growths occur in wild rabbits, and are transmissible to domestic animals of the same kind in which the growths are more often cancerous (63). Are some human tumors virus caused? Perhaps certain warts or condylomas are, for instance, those on the fingers, popularly supposed to be due to handling toads (63). The peculiar plantar warts are known to occur in kinds of "epidemics" in school children and others spread from gymnasium floors, shower baths, etc. The thorough work of Lucké with frog tumors is explained (64).

This leads to a general discussion of viruses as causes of growths and the following generalizations are rehearsed. Viruses are (*a*) procured from tumors of one general kind; (*b*) they are narrowly specific; (*c*) they must grow with cells; (*d*) they determine the kind of growth; (*e*) they increase as the tumors grow and are often recoverable; (*f*) many withstand drastic chemical treatment. They have great resistance to ultra-violet; (*g*) they can be absorbed and fixed by susceptible tissue; (*h*) they are antigenic (?) but not so when freed from tumor proteins; (*k*) they seem to act directly, making cells become tumor cells, i.e., they cause tumors as distinguished from bringing tumors into being; (*j*) most of them are larger than the smaller of the viruses causing other conditions. A brief statement as to some of the differences between these and other viruses is included.

Again as a generalization, it is stated that it is very unlikely that the general cause of tumors is a virus or an infectious organism for if such be the case, then all growth must be considered due to viruses or organisms. In passing, it is pointed out that the controversy as to the livingness or non-livingness of viruses is interesting from a general philosophical point of view and students are recommended to be alert for new developments in the way of crystallization of viruses, immunological problems, etc. (65).

Finally in relation to infection it is pointed out that cancer develops with some frequency in the scars, whether active or not, of syphilitic,

tuberculous and other lesions; but they also develop occasionally in the scars of old burns, and in x-ray or radium burns which are notorious for their predisposition to cancer. As one example of great clinical import, carcinoma of the tongue developing in a syphilitic is mentioned. On more than one occasion a syphilitic with a four plus Wassermann reaction of his blood has had an ulcer of the tongue treated for syphilis whereas it was in reality a cancer. This gives another opportunity, among innumerable others of emphasizing (*a*) the importance of biopsy, and (*b*) the fact that patients with any and all kinds of other diseases may develop cancer also.

11. *Biopsy and Other Methods of Diagnosis*

Apart from history, physical examination, Roentgen examination and procedures which use special instruments such as the cystoscope, proctoscope, bronchoscope, etc., the students are told, that there is no reliable test for cancer except biopsy. There are outlined to them a few tests of those which have been proposed and remained in vogue for longer or shorter times. Thus a number of tests done on the blood serum of suspected cancer patients, from an immunological point of view, are mentioned. Others utilizing chemical principles are detailed. The point is stressed that it is *early* cancer that must be detected if curative results are to be obtained. While it is possible that there are constitutional changes in individuals who are predisposed or are developing cancer, nevertheless none have been detected as yet. The further statement is made that biopsy is the foremost method of diagnosis and, given a properly chosen piece of tissue, an experienced pathologist should be able to detect malignancy or not in about 97 per cent of cases.

Methods of taking biopsy specimens are detailed with much thoroughness. The principles, it is repeated over and over, are: (*a*) Remove the suspicious tissue with a bit of the surrounding healthy area. (*b*) The part removed should be fixed immediately, preferably in formalin as a good general fixative, and should not be allowed to linger around the doctor's office until dried out and parchment-like. (*c*) Taking bits of necrotic tissue from the surfaces of growths is merely a waste of time. (*d*) Cutting off the tops of warts or papillomas is a certain way of being disappointed in the pathologist's report.

The students are told constantly that the microscopic diagnosis of malignancy in the majority of cases is made with a "low power lens and a high power brain," to use a favorite expression of Warthin.

Cellular detail has its place, but topographical microscopic anatomy is far more important in the average case. This is especially true in early cancer, just when the diagnosis is of critical importance to the patient. It may be possible to establish a diagnosis of malignancy by carefully examining a single cell, but the students are told that this is not the ordinary, nor, in the opinion of their teacher, is it the best way to establish the diagnosis.

Punch, needle, and similar kinds of biopsy methods are more often disappointments than not. They are told that when malignancy is well established, the removal, by needle or punch, of a bit of the tumor will often give a positive diagnosis. They are warned that in very early malignancy it is easy to miss the tumor with a needle or a punch, and besides, in these cases especially, it is topographical microscopic anatomy which is decisive, and this cannot be determined from a bit of rolled-up, fragmented, lacerated and torn bit of tissue, even if the operator is lucky or skilful enough to place the point of the needle in the suspected spot.

In Part 2 of the course, in which clinical cancer is shown and discussed, methods of biopsy in special regions are detailed. Thus, for instance, it is taught that no breast tumor should be disturbed in a doctor's office. The patient should be brought to the operating room with permission signed for a radical operation if necessary. There the entire lump should be removed and subjected to immediate examination. If the growth proves to be benign, the incision can be closed and the patient sent back to her room rejoicing. If it proves to be malignant, without further ado, radical amputation can be performed, whereupon the patient is again returned to her room perhaps not as joyful but nevertheless somewhat so. If a patient must needs develop cancer, it is much better to have radical treatment instituted before the clinical diagnosis can be definitely established. Therefore, such a patient, with a beginning cancer of the breast, should rejoice that it needed excision and frozen-section microscopic examination for its diagnosis. The chances for complete removal are excellent indeed.

Some time is devoted to a discussion of frozen sections and their value. The following points are made. In the majority of cases the diagnosis can be established on gross examination. In a number of others a frozen section will be decisive. In 5 to 10 per cent of the tumors doubtful on gross examination, there will be doubt with the frozen sections. These latter vary from part to part, being easier of diagnosis in certain organs, such as the breast. Brief histories of a

number of mistakes which their teacher has made in frozen-section diagnosis are rehearsed. For instance, he failed to diagnose Hodgkin's disease in a huge gastric ulcer. He missed diagnoses of bone tumor, and so on.

Again and again is encountered the belief that the performance of a biopsy is dangerous and tends to help spread the tumor. The students are told that the answers are three in number: (*a*) the biopsy should be properly done with sharp instruments or with electric cutting devices which produce a minimum of trauma and pressure; (*b*) extensive studies in mice have shown that when biopsies are properly performed, the danger of spread is a vanishing quantity; (*c*) properly performed biopsies are very much less dangerous in spreading tumors than repeated manipulations of tumors for examination purposes. The students are told that in a tumor clinic, when an examination is necessary by palpation, one, or at most two, members of the tumor clinic are asked to perform this examination. To have a dozen or more students or physicians stand in a row and await their turn to massage a tumor of the neck, or breast, or anywhere else, is extremely bad practice. The examination should be gentle, concise and to the point, not fumbling, hesitating, and diffuse. The students are urged to develop "eyes on the ends of their fingers" in the words of Dr. John B. Deaver.

12. *Tumors in Lower Animals*

Tumors, unlike other diseases, occur in all living organisms, at least in all multi-cellular organisms. This broad statement seems justified, for every year brings forth reports of the discovery of tumors in more and more varieties of animals and plants. Horses develop tumors, and the notable example of melanotic sarcoma in old white horses is mentioned. Dogs are quite prone to tumors, in fact, all mammals. It is a question interesting and, of course, as yet unsolved, as to whether the incidence of tumors in human beings is less or greater than that, for example, in dogs. The students are advised to visit the pathological laboratory and museum at the Philadelphia Zoo where examples of tumors in various wild animals can be seen. Tumors occur in birds, in fish, and some have been found in invertebrates. Tumors are common enough in plants and in spite of the controversies among plant pathologists (there are almost as many such controversies among animal pathologists) as to what is a tumor, the fact remains that growths appear in plants which, allowing for the

peculiarities of plant anatomy, are tumors (66). These facts, it is told to the students, are interesting from several points of view. (*a*) Experimental material can be obtained almost anywhere and has been brought to a high state of precision in the mouse. (*b*) All of us constantly hear remarks on the cause of cancer by well-meaning, but highly speculative people—"The reason human beings have so much cancer is because they are too nervous, or they eat too rapidly, or they cook their food in certain utensils, or they eat too much canned food." The students are told to answer these, at least in their own minds if for reasons of politeness or strategy they cannot answer the individual directly, with "Oh yes, the reason sharks develop cancer, I suppose, is because they eat too much canned peas, or become too nervous" or whatever idea has been presented to them on that particular day.

Finally, the statement is made as a repeat from the considerations given above that abnormal growth is statistically and theoretically a much more probable state of affairs than normal growth. And since all living organisms grow and develop, it would be most surprising if they did not develop tumors. It seems to be one of the possible penalties which all living things face as the result of their being alive.

13. *More About the Effect of the Environment*

The influence of still other factors of the environment (than carcinogens, etc.) is now considered. Naturally, their effect on normal growth and development is considered first as a point of departure.

Semantics are again invoked. When is environment not environment? A molecule of oxygen in front of the mouth of an animal is certainly environmental to the animal. When it is breathed in, it descends into the lungs, there to enter the blood in loose combination with hemoglobin or perhaps to be dissolved in the blood serum. When it reaches a cell it diffuses into the interior of the cell and becomes incorporated perhaps, into some compound or other. Finally it becomes part of a molecule of carbon dioxide, whereupon it diffuses out from the cell, enters the blood, is carried perhaps as carbonate, is released in the alveolar air and finally escapes. At what point does the molecule of oxygen which we have been following cease to be environment and become organism? In brief, it is necessary, the students are told, to specify environment and qualify with an explanation,—environmental to organism, environmental to cell, or what?

Again we do not like to split hairs but it is essential to understand each other.

Discussion is begun by stating that internal factors define the direction of development and take part in it. External factors define the nature of development and intensify, or modify, internal factors. Differences in development due to dissimilar environments are called modifications.

In addition to the individual action of environment, it is interesting to consider its trans-individual action, of which the much debated question of the inheritance of acquired characteristics is a part. Without entering into details, a few remarks are made on this subject, as follows: The interplay of internal with external factors produces the result in development. But the internal factors react with each other as well as with the external factors. There is a definite unchangeable norm for this, inasmuch as otherwise there would be no relation between generations, no species specificity, etc. This norm, however, may be changed by an alteration in the reactions by which the norm is accomplished. But this produces another norm and another reaction. A number of possible modes of action may be postulated on the germ cells in the new norm undergoing its own, now new, reactions. Temporary modulations and permanent (mutations) changes may result. The old problem of Lamarch is being re-examined today in the light of these newer conceptions. Specifically, in relation to oncology, can incidences which might have caused cancer in a parent be transmitted as cancer propensities to offspring? We hasten to say that no such factors are known, but we will not be blind to future data which may bear upon this question.

Gravity is an environmental factor to which everything is subject. It acts in a general sense, but, as in everything, it pays to examine the possibility of specific influence. It helps orient free-swimming eggs in which there is an unequal distribution of yolk with the result that the vegetative pole faces downward. Since the type of cleavage depends to an extent on the amount of yolk and on its arrangement, it therefore affects cleavage. In later stages of development of certain animals, it helps determine the direction of growth such as in hydra and hydroids. All things considered, its effect is non-specific and of no very great importance.

By exaggerating the force of gravity with centrifugation, some interesting facts have been learned. It is possible to take young germs, cause rearrangement of their protoplasm, and even rearrangement of

their growing cells. It alters both direction and rate of cleavage and is thus another tool for studying potency. Apart from the details learned by this method, the same findings are recorded as other means have discovered. In spite of much rearrangement of the parts of early embryos, regulatory phenomena, intervene and, unless there is actual disruption normal individuals finally appear.

At this place it is thought interesting to tell of Mrs. Harvey's experiments in which, by centrifugation and subsequent removal of the nucleus of fertilized sea urchin eggs, it is shown that without nuclei they can multiply to about 400 or 500 cells and can even differentiate!! This only makes it a little bit harder, the students are told, as they are reminded of our views of the importance of the nucleus to the cell. At any rate, the adaptability of protoplasm continues its amazing story under these circumstances and no cancer has been produced (67).

14. *Domestication*

An important environmental factor is domestication. Wild animals and wild plants both show interesting phenomena as a result of what we broadly call domestication. The "balance of Nature" is often disturbed by man, sometimes with good results and sometimes also, with disastrous ones to him and to the subjects.

But in the domestication of plants and animals and other parts of his environment to his needs and wants, man is modifying himself probably even more than his beasts and pets and flowers. It is a task for the future to unravel the complicated effects of domestication on himself.

In the minds of many domestication is a serious matter in cancer. It is stated so often that wild tribes who "live in a state of Nature" do not develop cancer, or they develop it with much less frequency than domesticated, i.e., civilized (!) man. The stress and strain of modern living, the eating of foods which are "not Nature's food" are statements which often torment the doctor. What shall he say to them? One answer has already been pointed out under *Cancer in Animals*. Another answer is that wherever accurate studies have been made of cancer in so-called savage, or partly civilized, races, age by age, just as much cancer has been found as in civilized man. It may be that the distribution varies a bit. Thus Bonné shows that the Malays have very little cancer of the stomach, but much primary cancer of the liver, in contrast to the Chinese, Europeans, and others

who live in the same or similar environments (68). But as far as total cancer is concerned, there has been found very little less, if any, among non-civilized races than among the civilized whenever and wherever competent studies have been made. Perhaps Malays, who may be considered by some to be not completely civilized (?) have an inherited predisposition to cancer of the stomach according to the principles outlined when heredity was discussed.

15. *Diet and Cancer*

While on this subject, a word or two is said to the students about diet in cancer. They are reminded that ingested foodstuffs are subject to profound chemical rearrangement before they are absorbed from the lumen of the gastro-intestinal tract. They are reminded that cells exercise choice in taking up building blocks from the fluids surrounding them. They are reminded once more that intracellular metabolism is another factor in the final disposition of foodstuffs. It is difficult to visualize diet, per se, as a cause of cancer, unless perchance substances like ortho-amino-azo-toluol are ingested by cells. Perhaps some, thus far undiscovered substance, acting similarly, may be present actually or potentially in food. As direct evidence attention is called to the studies made some years ago, wherein the incidence of cancer occurring among various monastic orders were tabulated. It was found that, although certain orders ate no meat, and others had other dietary customs, the amount of cancer occurring among the members was about that of the general population (69). Hoffman's studies, culminating in a large book, are also mentioned. He found that patients who develop cancer ate just a little more than those who did not, but of everything, with nothing specific about it (70). Many other related phenomena are discussed in this book, but many new facts about nutrition in general have been learned since its publication.

16. *Temperature*

In considering the effect of temperature on growth and development we distinguish maximum, optimum, and minimum temperatures operating on growth, survival, and fatality.

Further, temperature effects may be divided into non-specific and specific. High temperatures, in general, increase the rate of chemical activity according to the well-known rule that for every increase of 10° C. the rate of chemical change is doubled with the hindrances im-

posed by conditions of entropy and temperature coefficients of compounds, etc.

The study of heat effects on embryonic growth have shown that there are four or five pacemaker reactions in control at different times, but details of these reactions are as yet unknown. Usually changes in the temperature alter the rate, but not the amount of end products in growth and development. It seems there is a certain quota of material and energy to do a given amount of biological work. The rate varies at which this quota is rearranged and expended. All the energy used by an egg in developing comes from inside the egg itself; none is absorbed from the outside no matter how hot the environment. In warm-blooded animals a stage is reached wherein their own heat-regulatory apparatus keeps the growth rate constant. Usually it begins activity some time after birth. The effects of heat and cold are more manifest at so-called "critical" times, especially at the time of the first cleavages and after gastrulation. The mortality is then very high and of those which survive, nearly one-third become teratomatous. Growth is stopped or prevented just at times when important inequalities in the developmental rates of parts are needed for harmonious consummation. It is not known whether increased temperature effects are on the mitotic or intermitotic periods. As a tool for growth research, changes of temperature may be used for separation of proliferative, and differentiating, and organizing phases.

In certain cases blastomeres have separated as a result of heat, with resulting twins. Exogastrulation leaves the intestines outside the body, or perhaps no intestines at all are developed. Some mutations have been observed in Drosophila kept at high temperatures. There are higher red blood cell counts due to inspissation.

All of these changes, interesting as they are, have not as yet yielded fundamental information on cancer. They have, as stated, given information on growth and development which some day will be of use in the cancer problem. The treatment of cancer patients by cold has not cured them but very interesting findings in general physiology are recorded in "hibernating" patients. Klinke kept cancer and normal cells at temperatures of —253° C. and they survived and grew (71) when thawed quickly.

17. *Light*

The effect of light depends upon the wave length. Ultraviolet may inhibit cell division. Large quantities kill, especially embryonal cells.

It is of importance in the formation of pigment, not only in such animals as butterflies, but in man himself. Its relation to melanoma is discussed and time taken for a short discussion of melanotic tumors. The students are told that melanomas, nevi, etc., are coiled tactile corpuscles; that if the hairs from a human mole are pulled out, they would be analogous to the tactile corpuscles of amphibians. Conversely, if hairs are placed in the tactile corpuscles of amphibians they would resemble mammalian moles (72). Melonomas should never be treated with irradiation, the students are told. They are not of the epiderm but of the dermis. When it is determined to remove such a growth, the excision should be wide and in depth should be taken to and include the superficial facia covering the underlying muscle. Serial sections from around the part removed will reveal if the lines of the excision have surrounded the growth. If not, immediate further excision should be made of the part retaining the growth. Some moles are precancerous, but the students are told that as prophets their abilities are questionable. Of one hundred individuals with raised, corrugated moles on given parts of their bodies, some will develop metastasizing tumors. If every mole from all the one hundred people is excised properly, then none will develop malignancy. The students are told that this is not a philosophy of which to be proud; they should be able to predict which of the hundred will develop malignancy. But it is the best that can be done at present.

It has been mentioned that the increased incidence of cancer of exposed parts of the skin of sailors, farmers, fishermen, etc., is related to sunlight, perhaps by way of cholesterol, phytosterol, carcinogens (73). Direct experiments which expose animals to ultraviolet light have produced tumors but comparisons to human tumors await better control of the variable factors (74).

Light, as a factor in normal growth and development yields many examples. Thus the testes in birds develop or undergo atrophy in tune to light. Red light is more stimulating than white; green light is inhibitory (75). The development of the eyes is influenced by light, as for instance, the lack of eyes in certain cave-living animals and the inhibition of development of the eyes of others in darkness, e.g., axolotl. This effect is partly direct and partly by way of functional activity. The color of various animals is influenced by light showing that it is a factor in the chemical differentiation of cells which extends to the whole organism at times, for different color-types of certain

pupae can be distinguished by differences in the chemical composition of their tissue and hemo-lymph.

Interesting though these facts are, it is necessary to hurry to a consideration of the effects of the short wave lengths of x-rays and radium. Probably more time should be devoted to this latter subject but the students receive considerable information in the Department of Radiology and so a few generalizations are all that are given.

18. *X-Rays and Radium*

First there are considered the expressions "radiation sensitive" or "irradiation sensitive" or "radiosensitive." By them, the students are told, are meant degrees of resistance or susceptibility that a tissue exhibits to the x-rays or to radium. To understand more of this concept the following generalizations are given: When the Roentgen rays (Roentgen, 1895), and then radium (radioactivity, Becquerel, 1896) were introduced as methods of treatment for cancer it was hoped that these agents would pick out cancer cells, deal them a mortal blow, and leave the surrounding healthy cells uninjured. When these fond hopes failed of realization, as they did fairly promptly after the introduction of this method of treatment, it became a question of slight differentials between normal and cancerous tissues which were found to require much experience in judging the dosage.

From the x-ray tube are given off radiations coming from the surface or deeper in the metal of the target. These radiations are classified roughly as "hard" and "soft." The somewhat specific effect by which a differential in sensitivity between normal and cancerous tissue is attained comes from all of the rays by virtue of the conversion of their energy into chemical changes. But more dependence in therapy is placed upon "hard" rays because they penetrate more deeply. The softer rays are more destructive and corrosive of everything because they produce chemical changes of greater magnitude per quantum of energy delivered and absorbed. Whether hard or soft rays are produced depends on the composition of the target, and particularly the voltage which is impressed across the tube. In general, the less differentiated the cells of a tumor are, the more sensitive they are to the rays. Thus neoplasms like lymphosarcomas, carcinomas composed of entirely undifferentiated cells, etc., are much more readily destroyed by the rays than, for example, adeno-carcinomas of the rectum in which the cells are differentiated even to producing mucus, and are gathered together in attempts to imitate normal glands.

In the case of radium the following considerations are apropos: The alpha particles given off from radium are not allowed to come in contact with the tissues, in fact they are held back by the containers in which the radium is kept. The second kind of emission from radium is called the beta ray. They are analogous to the cathode rays within the Roentgen tube which are negative electrons. They are highly destructive and corrosive. The third irradiations are the gamma rays which correspond to "hard" Roentgen rays. They are the ones which are usually used to exert what specific and differential effect there is. Thus again, if a tumor is said to be radium sensitive or resistant it means to the gamma rays, as a rule.

In practice sometimes the beta rays are completely "filtered", i.e., removed, by placing a thin sheet of platinum, gold, copper, rubber, etc., between the radium and the tumor. At other times, especially in superficial growths such as basal cell carcinomas of the skin, the filters are so chosen that smaller or larger amounts of beta rays pass through and attack the tumor directly. If unfiltered beta irradiation is allowed to reach tissues, whether cancerous or not, they are simply corroded as though they had been painted with sulphuric acid or concentrated caustic potash.

The output of energy from x-ray tubes and from radium can be measured very accurately by various devices but there are no methods of predicting in a given patient what the biological effects of a given, physically measured dosage, will be. Just as blondes are more sensitive to sunburn, so also are they, in general, more sensitive to Roentgen rays and to radium. Radiologists develop a sort of sixth sense in this regard and while they calculate mathematically the dosage, etc., they nevertheless rely on empiric experiences for the individual case.

Further facts brought out to the students are that the intensity received by the tissues varies inversely as the square of the distance, so that if the Roentgen ray tube or the radium is placed close to the skin, the skin itself will receive a large amount of dosage and the underlying tissues less in proportion. If it is desired to treat a tumor under the skin, the radium or the x-ray tube is placed at a greater distance. The difference, then, between the dosage received by the skin and the part, say three centimeters below the skin, is not nearly so great. To avoid burning the skin in treating deep-seated cancer, distances are calculated, angles arranged in cross-fire fashion so that the tumor under the skin, or in the depths, receives a maximum, while any one area of the skin receives a minimum of exposure.

No attempt is made to discuss with the students the controversies as to the relative merits of oft-repeated small doses, or single massive doses, or "saturation" technic, etc., since this is felt to be the province of the radiologists. If any of the students choose to become radiologists they must learn these things at first hand and through personal experience.

A few facts, however, about the biological effects of irradiation are chosen. It is stated that x-rays produce mutations, as observed in the work of Muller, Patterson and many others. Whether or not the taking of repeated roentgenograms will cause changes in the chromosomes of the sex cells of patients so that any subsequent children develop mutations is not answered, but that there is danger not only of this, but also of producing really serious malformations and death of the fetus is insisted upon by Spillman (76).

Lacassagne and Coutard (77) particularly warn against irradiating the ovaries of women even with small doses if the women are likely to become pregnant subsequent to the irradiation. Murphy lists 155 publications on this subject (78). His studies with Goldstein (79) show that about one of 59 children born of mothers who had previous irradiations showed anomalies. DeRenyi and Murphy (80) followed this with experiments on rats in which they found that many of the animals did not conceive when the irradiation was applied to the back in the ovarian region, but those that did had normal-appearing offspring. Other works are quoted, all of which evidently show that more data are needed.

Naturally, the action of radiation on living tissue cannot be covered in extenso because of the enormous field and wide range of subjects. The students are told that the effects are manifold and that no single explanation is sufficient, i.e., its effects on any particular substance like cholesterol or cysteine, on some particular organs or tissues, will not explain their action in toto.

It is quite certain that secondary electrons from x- and gamma rays are active, perhaps essentially, in the production of many chemical and biological effects. Much is due to ionization from these rays as well as from alpha particles. Thus, for instance, the effect on colloids depends on the charge. If the colloids carry positive charges they become coagulated; if negative, they become more stable. Sols change to gels depending on their charges and the dose of x-rays, and also on the age of the colloid. Probably all these changes are more physical than chemical. Colloidal particles have their charges neutralized by

the ions formed in the double layer around them. Irradiation disturbs oxidation and reduction. It reduces methylene blue, brilliant green and other dyes. Glutathione and sulphydryl are destroyed. It stops oxidation in carbohydrate metabolism at a dosage which allows glycolysis to proceed. Enzymes are destroyed by large doses of beta and gamma rays. Rates of autolysis are increased; proteins are "denatured." Beta rays are much more effective in producing these changes in cells but the x- and gamma rays have qualitatively the same influence though less in degrees.

As far as cells and tissues are concerned, irradiation alters cell membranes with resulting changes in permeability. With a given dosage in less radiosensitive tissues the mitochondria and Golgi apparatus are caused to degenerate but they recover. In the more radiosensitive tissues the changes are progressive and irreversible. Staining reactions, nuclear membrane and nuclear content are influenced, again depending upon dosage and secondary radiation.

Cells in mitosis are more sensitive, probably just before the visible prophase, when temporary instability and decreased viscosity allow more of the ionizing effects of the radiations. Collagen fibers swell. To produce effects in tissue culture relatively large doses must be used. It is thought, therefore, that the therapeutic dosages are active because of delayed effects. A line of cells of low vitality results which die after two or three cell divisions.

Lacassagne (81) believes that good results in malignancy are due mostly to mitotic changes in the nature of mitotic degeneration. There are increased blood destruction and diminished formation even to the point of aplastic anemia; thrombosis occurs, obliterating endarteritis arises with degeneration of the media.

The products of destruction are mopped up by autolysis and phagocytosis. Connective tissue grows in and a fibroplastic inflammation results in repair. One of the reasons for refractory healing in tissue heavily irradiated is lack of blood supply. It is impossible to tell in many such ulcers whether they are due to irradiation alone or whether there are slowly multiplying malignant cells in the edges and bases. The treatment is so radically different that the students are told to promptly settle the question by biopsy. It seldom pays to treat an irradiation ulcer for months or years. Plastic surgery should be recommended after a reasonable attempt to cause healing is made with intelligent treatment.

The students are finally told that the results needed in malignancy

are not obtained by a low dosage. It is not sufficient merely to spray a few rays or electrons into a tissue. A boy cannot do a man's work, and if it is determined to really treat a cancer with irradiation, sufficient dosage must be given. Ordinarily, over-irradiation, especially of the skin, is better than progressing cancer. This, as is clear, is a function of amount and time and reaction of the tissues. It is emphasized to the students that if they become practitioners they may be required to care for patients who have been heavily irradiated. General recommendations appear later in the text. Specific ones are mentioned at this point such as the fibrosis which is often progressive in the lung under the parts irradiated for carcinoma of the breast, or fibroplastic changes in the kidneys and ureters, or stricture of the rectum following heavy irradiation for carcinoma of the uterus which sometimes requires colostomy. As stated, further details are to be gotten from the Department of Roentgenology and are discussed in Part 2 of the course, when patients present themselves with these various problems (82).

19. *Influence of Certain Chemical Compounds*

From the mass of existing data on the influence of chemical compounds on growth and development choice has to be exercised, for, obviously thorough consideration could easily extend throughout an entire year. Falling back on general principles, the students are reminded of a number of facts and considerations. What we observe in this world is chemical substance composed of molecules, groups, atoms, electrons, etc. Differences in the sensations received by us and through which we distinguish various types of substance are conditioned by differences in the composition and patterns of the chemical entities. The composition and arrangement of living matter are never constant for any appreciable length of time, but in the midst of these changes there is maintained a skeleton or framework of definite and specific constancy. This has been called a "dynamic equilibrium" by some, and by others has been termed "steady state." But it is not a steady state such as exists in an inorganic mixture, which also may not be "steady." In living things there is organization, as has been emphasized, and furthermore there is a direction to this "dynamic equilibrium" in time, for living things grow old. The students are told that if there is such a thing as evolution leading to the slow or rapid emergence of new forms and parts, there must also be evolution of

chemical compounds. New ones have to be evolved for new functions (or vice versa) as organisms develop new characters.

When speaking of multiple, almost infinite, potency as a biological phenomenon, it was related to the almost infinite possibilities of chemical union resulting from the presence within cells of the kinds of chemical compounds known to be there. The students are asked to think through the following remarks:

If we believe that all living things arose from a mass of primeval protoplasm, we must believe that the original mass had in it all the potencies for producing the many different kinds of living protoplasm that exist today, including cancer; or we must believe in the inheritance of acquired characteristics and/or in spontaneous generation!

It is not difficult to visualize the possibilities of infinite variation and increasing complexity of chemical compounds, for the first mass of living protoplasm needed but very few groups to start an endless chain. Given —OH, —NH_2, —CH_3, and =CO and will anyone be rash enough to say how many compounds can be made? Add a little S, Fe, Cu, and P and where will it end?

In any chemical study of growth and development, it is necessary to distinguish clearly between substances normally present inside of cells and substances in their environment. Possible methods of studying the effect of one or combinations of several compounds on growth and development are: (*a* & *b*) the mechanical removal or addition of a few molecules of a compound from or to the inside of cells. These are not feasible at present for technical reasons. This leaves two other methods: (*c*) to add known amounts to the media in which the organism is growing; (*d*) to subtract from the media all, or part, of the compound. These latter are the two methods in ordinary use. But we cannot say that a given compound, if it produces a certain effect when added to the media in which cells are present, is the compound that did the trick. It may be that when the particular compound was absorbed it was instantly changed, or it produced rearrangements within the cell, or a whole chain of them, and these are actually at the basis of the observed phenomena. While the possibilities were known for a long time, it is only within the last few years that the introduction of "tagged" atoms into experiments revealed the amazing lability inside of cells to be far greater than the wildest imaginations of a few years ago could suppose. Atoms leave their molecules, groups separate, and flit about from compound to compound seeking new bonds and syntheses and analyses in bewildering fashion. Amino

groups with extraordinary fickleness desert one amino acid to attach to another with the greatest of ease. Even the carbon atom in the inorganic sodium bicarbonate has been found to reappear bearing its original "tag" in the organic substance glycogen! We are thus compelled to think in this wise: When a chemical compound is offered to cells, as an example, proline, differentiation is enhanced, but we do not know whether the proline itself produces this effect or whether it gives up parts of itself to other substances which in their turn give us the results. Indeed, the idea is stressed and repeated over and over that groupings such as the amino, the carboxyl, the pyrrolidone, and many others are of more importance than the compounds to which they are attached in initiating and maintaining the ever active changes which occur in the growth and development of living things.

In relation to chemistry in cancer the students are urged to again make several distinctions. Thus there is a chemistry of cancerous tissue as contrasted with normal tissue. There is a chemistry of body fluids in individuals with, and without, cancer. There is a dynamic chemistry and physics of cancer in which the key subject is intracellular metabolism. Much more is known of the degradation of proteins than of their synthesis. But, as was pointed out before, the cancer cell builds up its internal equilibrium differently from a normal cell, and studies of protein synthesis are badly needed in this phase of cancer knowledge.

There are then discussed several details of intracellular chemistry, e.g., those experiments dealing with oxidation and reduction. Warburg's experiments are cited. The opinion is expressed that since oxidation and reduction are such universal cell activities, it might be better to add to the hunt experiments more characteristic of cancer cells, i.e., in protein metabolism. The students are told not to become discouraged at the difficulties of such a program. A start must be made some time. There are leads from test-tube chemistry which can be followed and perhaps one or another, or several of the students would like to engage in this work.

But to return to chemical substances and their effect upon growth and development. As an example of the effects of salts it is stated that in the absence of calcium salts the blastomeres in ova of sea urchins separate from each other as they do also when no sodium or potassium salts are present. This is a convenient way of producing twin sea urchins, or even six or eight, from one fertilized ovum. Excesses of lithium cause evagination of the archenteron producing an exogastrula.

Other salts produce the same effects. During later development, similar procedure give rise to open neural tubes, to clefts, and to a veritable spina bifida. In other cases no intestines are developed. Cyclops appears in the presence of excess magnesium salts.

These effects are sometimes general and sometimes specific. While teratomatous structures and various anomalies occur, cancer itself has not been produced in this way except with zinc chloride, etc., as mentioned under Carcinogens.

A few considerations of the effects of organic compounds, or amino acids, are chosen as examples. This is done for several reasons: (*a*) proteins are the most characteristic substances of living protoplasm and are composed of amino acids; (*b*) amino acids are universal in their distribution, i.e., not all are present in all animals and plants, but some are, and many different combinations can be made of them. A detailed account of methods of experimentation is given and finally the results, just as in the sulphydryl experiments and for the same reasons. The generalization is made that each amino acid has its own particular part to play in some phase or another of growth and development. Those of similar chemical composition, or those which are readily transformed into others, or yield the same groups give similar results. Thus, the pyrrolidone configuration influences differentiation and the imidine grouping is important in organization (83).

The point is stressed that a basis of comparison for cancer is being built up in this way. For instance, the cancer cell does not differentiate correctly. As stated, amino acid studies have shown that the pyrrolidone group, such as in glutamic acid, aspartic acid, proline, and hydroxyproline, has effect on the differentiation of normal cells. The hundred-year-old question of whether the cancer cell fails to differentiate correctly because it does not have access to the correct building blocks or that it simply cannot differentiate even if it has the correct building blocks, has now been answered. Given any amount of pyrrolidone the cancer cell cannot differentiate. This is again, the students are told, evidence of "somatic mutation" in the sense that those words have been used before.

20. *Trauma and Cancer*

This important subject is treated quite conservatively. The students are told that, in general, trauma is not a "cause" of malignancy, but that very occasionally a relationship may be present. Of all forms of malignancy there are two which are more frequently asso-

ciated with trauma than others; they are bone tumors and testicular malignancies. As stated under infections, etc., occasionally a malignancy will arise in the scar of an old trauma, more particularly those due to burns and those due to the scars of old infections of tuberculosis and syphilis.

The standard criteria are then rehearsed: (*a*) there was no tumor present in the region before the trauma; (*b*) the tumor arose in the exact region of the trauma; (*c*) the trauma was adequate; (*d*) a reasonable time lapsed between trauma and the development of the tumor—for point of departure this time is set at from four weeks to two years; (*e*) it must be proved that a tumor is actually present and this is best done by biopsy.

The tumors arising in aniline workers, tar workers, etc., have been mentioned. In the case of other so-called industrial cancers, the students are warned to be extremely careful in pronouncing judgment. Scientifically, the burden of proof is on the side which claims that a given cause produced the malignancy. Medico-legally, many times the patient, or the patient's relatives, since often the patient is dead by the time the case comes up for trial, are given the benefit of the doubt. A number of stories are told of their teacher's experiences in this subject, varying from perfectly ridiculous cases in which a broken fourth rib on the left side is said to have caused a carcinoma of the pylorus, and a case in which a bump resulting from an automobile hitting a rut in the road caused a perfectly benign bleeding from piles to turn into cancer of the rectum, to better authenticated and probable cases of severe trauma to bones or testes followed by malignancy.

The students are warned not to talk too much to patients or relatives in such cases and under no circumstances to enter court as an "expert" witness unless they have primed themselves thoroughly in their subject and in the case under discussion.

During the clinical part of the course several such patients usually are presented and the opportunity taken for further discussion.

C. Clinical Practice

In this part of the course, patients presented to the students are used as texts in two ways: (*a*) the individual peculiarities and specialities of the particular patient and (*b*) the relationship of that patient's disease to any and all of the general principles discussed before.

Before patients are presented, however, a number of considerations in the patient-physician relationship are discussed. The students are told never to forget that patients with malignancy have a serious, fatal disease. Very often indeed the fate of the cancer patient depends upon the first doctor who sees the patient. This dependence includes not only actual handling of the physical disease but of the patient's mental attitude as well. That patient is an exception whose mental make-up can be sized up accurately enough at the first meeting to decide the important question, *Shall the patient be told?* In fact it cannot be decided always after many consultations. Generalities will not do; in every individual patient it must be decided afresh as to whether he or she should be informed of the disease. Each case must be decided upon its own merits. If the patient is of such temperament that the knowledge he has the disease will give rise to a feeling of fight, a victory is won. For family, legal or business reasons, the patient must ofttimes be told. If on the other hand the patient is one in which knowledge of the diagnosis will produce a profoundly hopeless view he should not be told. In very early cases the decision is usually to tell the patient; in advanced cases, usually not. Under any and all circumstances, however, some responsible member of the family must know the truth. Care should be taken in the choice of that member; an uninstructed or careless family and well-meaning friends (!) can undo in one sentence or even a look all the mental good that has taken weeks to accomplish.

The above remarks about telling the patient must be tempered by the nature of the procedure for the elimination of the cancer. Contemplation of the removal of breasts, arms, legs, pelvic organs, etc., is a shock to patients. They must be told what the procedure is to be; the adaptability of human beings should be stressed and how mental, physical, and economic compensation can be achieved by the will to do it. There are literally dozens of ways, the students are told, in which they can be of help in the crisis.

Many patients are nervous and apprehensive when presented before the students. It is my custom to ask whether they object to helping me teach my students to be better doctors than I am; the next generation of doctors must be better equipped to take care of patients than we are. Not once has there been objection and in most cases a decided willingness to help. Undue exposure is never tolerated. Carcinomas of the cervix cannot be seen by whole classes, and in such cases, or similar ones, no more than two students see the patient's lesion

before the clinic. The students are told to describe what they saw in such vivid terms that their classmates will not fail to obtain a mental picture of the lesion!

While the history is being recited, the patient is asked to listen and check the statements. This simple request is sufficient in most cases to turn patients' anxiety into other channels and often important details are added to the history. Most patients are not cheerful when they enter the clinic. It takes but a second or two to discover any special interests or hobbies. The students are told that a patient should not leave the clinic without a smile, and if this is accomplished, as much of a victory is won as when the diagnosis itself is made.

The student body is asked to formulate questions to the patient but they are told over and over to be careful in their framing. The patient must not leave the clinic with a diagnosis of cancer absolutely settled. Most of them suspect it but there must be a reasonable doubt in the patient's mind. As stated, this doubt will be clarified or not when later studies reveal the mental make-up of the individual. I may be criticized, but I "lie like a gentleman" at times.

The method of procedure from the technical point of view is as follows (84): As an instance, we have a patient who has discovered a lump in the breast. The history is recited and description given of the physical examination. Questions are asked and the answers noted. The patient is dismissed. Again and again it is urged to the students that they "develop eyes on the ends of their fingers"; that examinations be brief and pointed and not diffuse; that squeezing and manipulating cancers are excellent ways of disseminating emboli and spreading the tumor. It is far more dangerous than a properly performed biopsy which should have no danger. There are then rehearsed the embryology of the breast, some of the comparative anatomy, the normal anatomy, and the histology of the organ as much as possible in such biological relations as potency, determination, differentiation, etc. There is no such thing as a normal breast; there are many different kinds of normal breasts depending upon age and sex, endocrine relations, etc. The physiology of the breast is discussed along with a bit of its chemistry. Various pathological conditions are outlined ending with a consideration of the clinical differential diagnosis of lumps in the breast.

The students are told that none of them will ever develop sufficient clinical skill to distinguish between benign and malignant lesions in the stages in which we want to see them, i.e., when they are very tiny.

They need not have studied medicine to diagnosis cancer of the breast when it is well advanced. Our grandmothers were expert at this feat. Since they cannot distinguish clinically very small lesions and since the diagnosis is of such importance to the patient they must think of other methods to make the diagnosis. To think of biopsy and lumps and ulcers as one thought process should be second nature. Lumps should be removed but not in their offices. In the operating room the diagnosis is established by combined gross and frozen-section examination whereupon the proper treatment can be instituted.

Irradiation before and after operation for cancer of the breast is discussed. Their teacher's opinion is that we have not accumulated sufficient data to decide, but certainly, the students are told, irradiation before operation should not be done for tiny lumps for they may not be cancers, and if irradiation is used, as stated above, it must be used with sufficient dosage to obtain effects.

The after care of the patient is then discussed in general terms, the necessity for follow-up, the common places to hunt for developing residual growths, the common complications and methods of circumventing or treating them.

Throughout the whole course the word "recurrence" is used only to be condemned. "That which was not removed or destroyed will continue to grow." Recurrences, the students are told, are merely continuations of growth of tumors which were not removed or destroyed in toto. Pedagogically, it is thought that this will bring vividly to their attention the prime necessity of cancer treatment, i.e., complete removal and/or destruction of the growth.

It may be that later the patient will return to the clinic for a follow-up examination depending on the time relations.

In this way all of the common forms of cancer are covered during the year. Innumerable points appear and discussion follows wherever it seems to lead logically. During the past year the following cancers have been studied: those of the breast, uterus, thyroid, prostate, skin, tongue, mouth, rectum, bones, lungs, larynx, and others. In addition Hodgkin's disease and the leukemias received attention. Naturally, non-tumorous lesions also are chosen to help in fixing differential diagnostic points. Lantern slides and the projector for histological preparations are used freely.

Effort is made to find patients with early lesions. Unfortunately, this is not always possible. But at least the opportunity is seized for repeating remarks about delay time. Never has a patient with an

older tumor appeared who has not been asked by one of the students, "Why didn't you see your doctor earlier?" Some patients say they did visit a physician immediately after discovery of the lesion. The students discuss the reasons for the doctor's delay, ofttimes in vituperative terms—after the patient has left. In many cases, patients with older lesions express regret at their delay. All are promptly enlisted as missionaries to spread among their friends the gospel of early attention. Some are given literature for distribution. All are made to feel that their trouble, even if serious, nevertheless can be turned to some good account.

1. *Diet for the Cancer Patient*

While diet, per se, is a problematic factor in the production of human cancer, it is nevertheless an important consideration in the general handling of the cancer patient. The students are advised to consult in this matter in detailed terms with their patients and the relatives. For tissue repair, blood regeneration and maintenance, proteins should be emphasized; for energy, carbohydrates and fats; for replacement, building new tissues, and as helps in regulating bodily processes, minerals should be emphasized. Vitamins are not to be neglected. The appetites of cancer patients are ofttimes fickle and poor, apart from the special difficulties of those with carcinoma of the stomach, esophagus, tongue or mouth, etc. These patients require special attention. Unless there is good reason, foods of which the patient is particularly fond should not be prohibited. The judicious use of a cocktail, of sherry or other wines before meals is a useful adjunct in many instances. The three-meal-a-day habit is not sacred, and four or even six small meals may be served to those patients who complain that they feel hungry but are quickly satisfied by small amounts. The old tricks, which are often forgotten, are told to the students; the smell of appetizing food as it is cooking is a stimulus to the appetite; place more on the tray than the patient is expected to eat—most people are inclined to eat more to avoid waste; good company and interesting conversation are helps. About 2000 calories per day should be chosen from ever-changing combinations, prepared in many different ways, and served as attractively as possible.

The students are told that attention to these details is especially important, not only before and after recovery from extensive operative procedures, but also before and after irradiation therapy.

2. *Nursing Care*

Cancer is a chronic disease and the patient may be unable to afford the services of a graduate nurse except to tide over difficult periods. Nursing care over long periods is delegated to "practical" nurses or to the members of the family. Detailed instruction from the doctor is much appreciated by both the patient and the relatives. The proper care of beds, the judicious placing of pillows or sand bags removes small but persistent annoyances and relieves tired, strained muscles. The care of dressings requires instruction; enemas can be very painful if given unskilfully; the maintenance of good skin tone is important; chafing and rawness add to the patient's burdens. Proper soaps should be chosen and oily preparations for rubbing into the skin are often better than the commonly used alcohol. Methods of removing odors can be told to the caretaker of the patient. These and many other small but important details should not be overlooked.

3. *Care of Cancerous Wounds*

It has been mentioned that the differential diagnosis between a non-healing, non-malignant ulcer and one which has tumor cells in it cannot always be made clinically. Biopsy should be prompt whenever there is the faintest doubt. Details of the care of such wounds are left to the surgical department for exposition. In the oncology course, the attention of the students is drawn to the necessity of their proper management.

4. *Sleeplessness and Pain*

It is well to make a distinction between sleeplessness due to worry, etc., and that due to actual physical pain. Appropriate measures for sleeplessness include psychotherapy, the removal of worry due to financial and family affairs as far as possible, attention to the care of the bed, proper ventilation, proper temperature of the room, absence of street noises, etc.

The students have been made familiar with drugs. They are told that, in general, milder drugs should be used at first for the control of pain, but that the sheet anchor for the intractable pain of advanced cancer is morphine. In considering the proper dosage for such patients, the only answer is that enough should be given to deaden pain. Again and again, however, the students are warned to be sure of their diagnosis before morphine is given and addiction risked. Many a

cancer patient has pain from other, possibly non-fatal, causes. This must not be forgotten.

Throughout all of this discussion in relation to the general care of cancer patients it is emphasized that the good doctor may make mistakes or miss diagnoses, but never because he fails to listen to the story of the patient, to make physical examinations, and call to his aid such special help as may be necessary in a given case.

5. *Quacks*

Some of the patients who are presented have been treated by quacks. Most students exhibit a degree of indignation at quacks untempered by the experience and knowledge which they acquire later of the frailties of human beings in health matters.

Francis Carter Wood's classification is given to the students: (*a*) well-meaning and sincere but untrained and ignorant folks who are inspired to think they are heaven-sent missionaries to rid the world of the curse of cancer; (*b*) well-meaning and kindly folks who know of some remedy, ofttimes discovered by an old Indian, or gypsy, or other mysterious person; (*c*) well-meaning but ignorant folks who have discovered a cure and are willing to part with it—for a price; (*d*) smart people who know they have nothing of value but understand how to "sell" things and are perfectly willing to trade on the despair of cancer patients and relatives for as big a fee as the traffic will bear—many of these have worked as orderlies, or even as janitors, in hospitals, etc., and have picked up a smattering of the lingo which they use very effectively; (*e*) a few renegade doctors appear from time to time, some of them with weakness of mind and judgment, and some with deliberate intent to deceive for profit.

The students are told that many cancer patients, at some time or other, consider such and such a new "cure" read about in newspapers, or magazines, or heard across the back fence from neighbors. Probably there have been several million "cures" for cancer discovered since the dawn of mankind. None of these has proved worthy and the probabilities that the "new" one which the patient or relatives want to try is effective is certainly at the famous mathematical vanishing point. But if patients still insist upon trying such remedies, they can be told that they had better try one of the "new" ones because they, at least, have not as yet had time to prove themselves unworthy.

The most damning testimonial that can be claimed for a cancer

cure is that it will "relieve pain and make the patient more comfortable," for this immediately reveals its unspecificity, its appeal to psychotherapeusis, and places it in competition with the tried and true pain relievers, of which there are many.

Many of the pastes, ointments, and similar preparations which are used undoubtedly will corrode and destroy superficial cancers. If patients insist upon having these used upon them, the students are told that a good answer is that we no longer ride on the backs of donkeys but use automobiles, etc., to travel distances. If the patients want to use such old methods as were in vogue in ancient times, it is their privilege, but modern methods are quicker, cleaner, less painful, less liable to secondary infections, and leave better cosmetic effects than salves, pastes, teas, or whatever. Besides the individuals who apply these pastes do not exercise their "art" for nothing and in the long run practically every patient who tries them finds that they cost far more than the accepted methods.

Pamphlets and reprints concerning quacks and quack remedies can be obtained from the offices of the American Medical Association. Copies of fraud orders issued by the United States Post Office against various cancer cures are readily obtainable. To some patients and their families these are convincing.

6. *Method of Rating Students*

During the course of the year, by means of discussions and observations of the behavior of the students towards the subject, it is possible to obtain a general idea of their ability. At the end of the year a written examination is given. Their teacher does not entirely approve of this but this is no place to air controversies relating to pedagogical methods of appraisal. A sample of the examination is given. They include both background and clinical questions and they are rather of the discussion type than the dogmatic yes or no type.

Answer (1) and (2) and Three Other Questions

(1) Outline a scheme of procedure for the diagnosis of lumps in the neck.
(2) Briefly, what is meant by differentiation of cells and its importance in oncology?
(3) Discuss the diagnostic features of carcinoma of the rectum.
(4) Discuss as for a lay audience the background of "diagnose and treat cancer early."
(5) Indicate the essentials of obtaining a biopsy specimen.
(6) Discuss the general management of a cancer patient.

7. *Function of the Hospital Tumor Clinic in Relation to Teaching Oncology*

Tumor clinics in hospitals are held usually once a week for an hour or more, depending upon the number of cases. Ordinarily, to this clinic are referred the more difficult diagnostic problems as well as the rarer cases. From the point of view of fourth-year medical students, it is good pedagogy to have attend small sub-sections consisting of not more than 10 students. They hear seasoned discussion and arguments. They hear the family doctor's point of view, for many times the family doctor presents his patient. They become familiar with the field of use of various specialized technical procedures such as cystoscopy, bronchoscopy, gastroscopy and others, along with their shortcomings and advantages. They hear presented points of view on treatment, chiefly those of the surgeon and radiologist. They may even be asked to present at the next meeting a short résumé of some particular phase of oncology.

This has the disadvantage of presenting "rare" cases, whereas, strictly speaking, it is the commoner conditions which should be emphasized to fourth-year medical students. Nevertheless it is an experience for them of great value.

The organization of the Tumor Clinic at Hahnemann Hospital consists of a Chairman who is a pathologist, a Chief of the Clinic who is a radium therapist, and Consultants in all of the specialties. The Clinic acts in a diagnostic and advisory capacity. Its recommendations are passed on to the respective chiefs on whose services the patients are entered for them to accept or reject, as the case may be. It also acts as a clearing house for various ideas of local as well as general origin and at present is interested in certain diagnostic tests for cancer. It maintains contact with the various cancer committees of local, state, and national organizations, and with the Wainwright Tumor Clinic Association of Pennsylvania.

This Clinic is an integral part of the Department of Oncology.

8. *Special Undergraduate Activities*

For the past three years a cash prize has been offered for the best presentation by a member of the senior class of any subject in oncology. Competition has been keen and very creditable papers have been presented. The subjects included some of biological interest, others of experimental cancer research, and still others of clinical

considerations. The former are reviews and correlations of literature; several of the latter have been first hand studies of patients.

Three years ago, an Oncological Society was founded with undergraduate members. It meets once a month and has adopted the following program. The history of a case occurring in the hospital is read by a second year student. The third and fourth year students attempt to diagnose the case, giving reasons for their statements. When the diagnosis is finally given, a first year student presents a brief résumé of the anatomy of the region thus, for instance, if the case turns out to be a carcinoma of the stomach, the second year students present résumés of the pathology and physiology of the stomach. A third year student then concludes the program by rehearsing the symptoms as they should have been according to text book descriptions. The final event of the evening meeting is a talk on some subject in oncology by an individual other than a member of the Faculty of Hahnemann Medical College and Hospital. The latter is done so that different points of view are presented than those with which the students have become familiar from their own teachers.

The members of the Society conduct what they call a "Model Tumor Clinic." One of the members becomes a patient, another one the family doctor, others become surgeons, pathologists, radiologists, internists, etc., for the evening. The case is carefully presented, the diagnostic possibilities are discussed, the laboratory procedures indicated, and finally all of the data summed up for the complete diagnosis. This is a carefully rehearsed performance and is given in the Auditorium for attendance of the rest of the students and any others interested.

By means of this Society and its activities it has been found that foci of special activity are generated in each of the classes for furtherance of oncologic interest.

D. Summary and Conclusions

The reasons for the establishment of a course in Oncology are given in the body of the paper. They include such factors as the scattered nature of present day teaching in this subject and rehearsals of why Oncology is important enough to deserve special attention. There is a skeleton outline of a type of course which can be given to fourth year students.

It is entirely feasible and useful to organize such a course in a formal Department of Oncology. Various adjuncts in teaching, such

as tumor clinics, essays, special undergraduate activities, etc., are detailed.

It is impossible for me to acknowledge by name all of the many friends who have discussed these matters with me. They include biologists of various kinds, surgeons, radiologists, internists, specialists of all medical orders, my own teachers, and many persons particularly interested in oncology who have especially encouraged this experiment. Last but not least, I add my thanks to many students not only in my classes at Hahnemann Medical College and Hospital, but also in the University of Pennsylvania and the Women's Medical College of Pennsylvania. To my colleagues in research at the Lankenau Hospital Research Institute I also express appreciation for their patience in leading me into the mazes of theoretical biology, biochemistry, organic chemistry, and into certain applications of these disciplines as they apply to growth problems. In various official capacities, I have been in intimate contact with many practitioners and thank them for the ideas they presented, and for telling me of their difficulties, and of their methods of surmounting them, in the practical field of tumor diagnosis and therapy.

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ERRATUM NOTE

In the paper entitled *The Eruption of Deciduous Teeth* by M. Robinow, M.D., T. W. Richards, Ph.D., and Margaret Anderson, M.S.P.H., on page 130, vol. 6, the lines in the correlation table were omitted. The table should read as follows:

TABLE 3

INTERCORRELATIONS OF AGES AT WHICH TEETH ERUPTED

	U_1	L_1	U_2	L_2	U_3	L_3	U_4	L_4	U_5	L_5	*HT.*	*O.C.*	*Wk.*
U_1													
L_1	.66												
U_2	.82	.63											
L_2	.59	.58	.49										
U_3	.73	.68	.70	.72									
L_3	.69	.67	.67	.69	.92								
U_4	.57	.51	.47	.40	.63	.58							
L_4	.53	.49	.46	.49	.74	.71	.74						
U_5	.50	.32	.46	.32	.61	.55	.64	.61					
L_5	.52	.43	.49	.38	.62	.57	.75	.67	.86				
Ht.	.28	.25	.32	.25	.24	.25	.22	.26	—.01	.03			
O.C.	.19	.17	.10	.18	.09	.18	.25	.23	—.03	—.07	.31		
Wk.	.18	.16	.25	—.01	.13	.05	.10	.11	.09	.14	.03	.10	

Ht. = Age at which height of the average 18-months-old child was reached.
Wk. = Age at which the child began to walk unsupported.
O.C. = Age at which the child had developed the number of ossification centers seen in the X-rays of the average 18-months-old child.
Abbreviations for teeth as in Table 1.